EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Chromium (hexavalent compounds)

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code Section 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. Chromium (hexavalent compounds) has been included on California's Proposition 65 list of chemicals known to cause cancer since February 1987 and has a No Significant Risk Level of 0.001 μg/day (by inhalation). This document by OEHHA addresses the reproductive toxicity of these compounds, and provides information on whether these compounds should be identified as known to cause reproductive toxicity under Proposition 65.

One of the mechanisms by which "a chemical is known to the state to cause cancer or reproductive toxicity [is] if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant (DART) Identification Committee of the OEHHA's Science Advisory Board (Title 27, California Code of Regulations, Section 25302)[1].

This document provided the DART Identification Committee with information relevant to the reproductive toxicity of chromium (hexavalent compounds). It includes literature available to OEHHA prior to September 5, 2008, the date on which the draft was made available to the public. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee was held on November 20, 2008, in Sacramento, California. Following discussion and Committee deliberation, the Committee determined that chromium (hexavalent compounds) "have been clearly shown through scientifically valid testing according to generally accepted principles" to cause developmental, female reproductive and male reproductive toxicity. Chromium (hexavalent compounds) were listed under Proposition 65 for these endpoints effective December 19, 2008.

[1] Formerly Title 22 California Code of Regulations, Section 12302

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A. ABSTRACT

Hexavalent chromium compounds are those that contain the metallic element chromium (Cr) in its hexavalent (positive-6) valence state. In this document, these compounds are denoted chromium (hexavalent compounds), or Cr(VI) compounds.

In the environment, Cr(VI) may be reduced to the trivalent form (Cr(III)), although hexavalent forms may also persist: Cr(VI) compounds occur as contaminants in ambient air, drinking water, soil, house dust and food. Occupational exposures to Cr(VI) occur, for example, during the production and welding of stainless steel, in chrome plating operations, and in applying paints and coating containing Cr(VI) compounds. Hexavalent chromium has been shown to be absorbed during occupational inhalation exposures. Gastrointestinal absorption of Cr(VI) is low (estimated to be less than 5% in humans), but greater than for Cr(III). The consistent finding of lung cancer in workers exposed during chromate and chromate pigment production and chromium plating has led to the identification of Cr(VI) compounds as a known human carcinogens by a number of authoritative institutions; there are also consistent findings of cancer for Cr(VI) in animal experiments. Recently, in experiments conducted at the National Toxicology Program (NTP), oral exposure to Cr(VI) (as sodium dichromate dehydrate) resulted in clear findings in both sexes of cancers of the oral cavity (rats) and small intestine (mice).

Multiple studies of the developmental toxicity of Cr(VI) in experimental animal models such as rats and mice have demonstrated decreased viability of the conceptus (both pre- and post-implantation), decreased fetal weights and crown-rump lengths (CRL), changes in placental weights (decreased or increased), and increased frequencies of external and skeletal anomalies. These effects were reported both in studies where exposure of the dams occurred during gestation and in studies where exposure of the female animals ended immediately prior to mating. Epidemiological data on Cr(VI) exposure and developmental toxicity are sparse. An epidemiological study of ecological design did not find increases in total congenital malformations in neighborhoods residing close to the chromium wastes, but this study had a number of methodological limitations. A case-control analysis of a study designed to look at behavioral factors and later pregnancy outcomes (congenital anomalies, stillbirths or neonatal deaths) found no significant association of chromium levels in the drinking water. The odds ratio (OR) for musculoskeletal defects was decreased with borderline significance, and risk for still births was elevated in both the crude and adjusted estimates, but did not achieve statistical significance. The linkage to water quality data was secondary to the examination of behavior risk factors.

Effects found in studies of female reproductive toxicity in mice and rats include: lengthening of the estrous cycle in both rats and mice, decreased mating and fertility indices; decreased numbers of corpora lutea, implantation sites, and live fetuses/litter; and increased frequencies of pre- and post-implantation loss. Ovarian alterations at the ultra-structural level were also reported. Human data on female reproductive toxicity are limited to poorly reported studies of factory workers exposed in Russia. Pregnancy

complications and toxicosis are reported to be substantially elevated in the most exposed, but the studies are too poorly reported to make firm conclusions.

Multiple studies in multiple animal species by multiple routes of exposure have shown adverse effects on some aspect of the male reproductive system, regardless of species or whether Cr(VI) was given in drinking water, in feed, by gavage, or by intraperitoneal (i.p.) injection. Of 16 animal studies found in the literature, only one did not find male reproductive effects. Observations included histopathology findings, altered sperm parameters, altered testicular biochemistry, altered sexual and aggressive behavior, altered weights of testes, epididymides, and/or other accessory organs, decreases in testicular protein, DNA, and RNA, and decreased serum and/or testicular testosterone. Morphological damage, observed by light microscopy and/or at the ultrastructural level, was described following Cr(VI) exposure in non-human primates, rats, mice and rabbits. Specific effects reported included phagocytosed sperm, disorganized seminiferous tubules, damage to the epididymal epithelium, damage to Sertoli cell membranes, decreased seminiferous tubule diameter, atrophic seminiferous tubules with loss of spermiogenic epithelium and edema of testicular interstitial tissues. The human data are generally consistent with these findings of male reproductive toxicity in animals. A number of studies examine the potential impacts on male reproductive health related to exposure to stainless steel welding with its Cr(VI) fumes. In addition, one study considered these effects in men working in a chromate factory. These studies examined effects on semen quality, fecundability, infertility, and male-mediated spontaneous abortion in couples that include a male stainless steel welder. A number of studies found associations of sperm quality effects and indicators of Cr(VI) exposure, including effects on sperm count, mobility and morphology. In addition, a significantly increased rate ratio for spontaneous abortion with current paternal stainless steel welding was found. While not all studies showed positive results, study limitations especially with regard to establishing groups with clear differences in exposure status appear to explain most of the negative findings. The studies with the higher exposures or better designs found increased risks of adverse outcomes in men with Cr(VI) exposure.

B. INTRODUCTION

B.1. Chemical structure and characteristics

This hazard identification document pertains to chromium (hexavalent compounds).

Hexavalent chromium, or (Cr(VI)), compounds are those that contain the metallic element chromium (Cr) in its +6 valence (hexavalent) state. In this document these compounds are denoted as chromium (hexavalent compounds), or Cr(VI) compounds. Chromium has six oxidation states. The hexavalent state is one of the three most stable forms in which chromium is found in the environment (U.S. EPA, 1988). The other two of these forms are the 0 (metal and alloys), and the +3 (trivalent chromium, Cr(III)) valence states.

In nature, chromium generally occurs in small quantities associated with other metals, particularly iron. Its atomic weight is 51.996. Hexavalent chromium, in contrast to the trivalent form, exists as highly-oxidizing species (IARC, 1990). As noted by NTP (2008), Cr(VI) is usually "present in complexes with halide (chromyl chloride) and oxygen ligands (chromium trioxide, chromate, dichromate)." There are numerous Cr(VI) compounds. Some examples are potassium chromate, dichromate, sodium chromate, chromium trioxide, and lead chromate. Hexavalent chromium compounds can vary considerably in their water solubility and other physical properties.

Most chromate (Cr(VI)) results from man-made production, as the form is rare in nature (Barceloux, 1999). Hexavalent chromium reduces readily to Cr(III); the rate increases with decreasing pH (Barceloux, 1999). The NTP (2008) notes that "Cr(VI) is easily reduced to Cr(III) in acidic solutions containing organic molecules such as proteins, DNA, or glutathione." Glutathione is also capable of reducing Cr(VI) at neutral pH at a slower rate than under acidic conditions.

B.2. Use and exposure

Chromium metal is usually produced by reducing the chromite ($FeCr_2O_4$) ore with aluminum (Weast et al., 1988). Chromium is used to harden steel, in the manufacture of stainless steel, and in the production of a number of industrially important alloys (Weast et al., 1988). Chromium is used in making of pigments, in leather tanning and for welding. Chromium plating produces a hard mirror-like surface on metal parts that resists corrosion and enhances appearance.

The general public and communities have been exposed via air to Cr(VI) through manufacturing emissions, its use as an anticorrosive agent in cooling systems, chrome plating, and combustion releases; for example, in fly ash from power plants and cigarette smoke. The California Air Resources Board, consequent to the identification of hexavalent chromium as an Toxic Air Contaminant (OEHHA, 2000), has taken a number of steps to reduce the public's exposure to Cr(IV) in air, including a prohibition on its use

in cooling towers and development and enforcement of standards on chrome plating operations.

In the environment, Cr(VI) may be reduced to the trivalent form (Cr(III)), although hexavalent forms may also persist: Cr(VI) compounds occur as contaminants in ambient air, drinking water, soil, house dust and food. Trivalent chromium and Cr(VI) are inter-convertible in the environment. Oze et al. (2006) note the occurrence of naturally occurring Cr(VI) in ground and surface waters, and its generation through natural processes. Mechanisms for its generation from Cr(III) from serpentine-derived soils and sediments and migration into water sources have been described by these authors. Serpentine, the California State Rock, is prevalent in central and northern California. It is unclear how much exposure in the State to Cr(VI) in drinking water results from such processes.

Contamination of drinking water with Cr(VI) also has resulted from industrial uses, such as in plating operations. In water that is rich in organic content, Cr(VI) is most likely to react quickly with reducing agents to form Cr(III) (U.S. EPA, 1988). However, Cr(VI) may persist in water as water-soluble complex anions. Legacy contamination of drinking water sources from previous uses in cooling towers and in manufacturing continues to result in site clean-up orders in the State.

Virtually all foods contain some chromium, ranging from 20 to 590 μg/kg (U.S. EPA, 1985). The foods with the highest levels of chromium are meats, mollusks, crustaceans, vegetables, and unrefined sugar (U.S. EPA, 1985). Trivalent chromium tends to form stable complexes with organic and inorganic ligands, and is presumed to be the form found in foodstuffs due to the presence of reducing agents in food (U.S. EPA, 1988; NAS and FNB, 2000). There are debates over the essentiality of Cr(III) (Sterns, 2000), and its use as a nutritional supplement in sports medicine (Vincent, 2003) and to treat insulin resistance (Gunton et al., 2005). The National Academy of Sciences (NAS) did not find sufficient evidence to set an Estimated Average Requirement (EAR) for chromium, but did set an Adequate Intake (AI) for chromium of 35 μg/day for "young men" and 25 μg/day for "young women" (NAS and FNB, 2000). Most recently the Institute of Medicine (IOM, 2004) reviewed the safety of chromium picolinate, the nutritional supplement form of Cr(III) and found "there is neither consistent evidence of reasonable expectation of harm from chromium picolinate nor sufficient evidence to raise concern regarding the safety or toxicity of chromium picolinate when used in the intended manner for a length of time consistent with the published clinical data."

Workers experience the highest exposures to Cr(VI) through chrome plating, chromate production and stainless steel welding (NTP, 2008). Usually the route of occupational exposure is inhalation or dermal contact.

B.3. Metabolism and pharmacokinetics

The NTP (2008) has recently synthesized and succinctly summarized information on the absorption, distribution, metabolism, and excretion of chromium and chromium compounds. Much of the text below is abstracted directly from the NTP document.

Absorption

Chromium and chromium compounds are found to be absorbed after oral, dermal, or inhalation exposure. Most studies of absorption of Cr(III) or Cr(VI) after oral administration to rodents found that only 1% or 2% of the administered dose is bioavailable, whereas similar studies with humans report somewhat higher numbers, particularly for Cr(VI). It is thought that Cr(VI) is poorly absorbed when ingested due to its rapid reduction to less soluble Cr(III) in the presence of food and the acidic environment encountered in the stomach. Trivalent chromium is less efficiently absorbed than Cr(VI) compounds, and this is attributed to a difference in their respective methods of transport into cells. Hexavalent chromium enters cells by facilitated diffusion via nonspecific anion channels, while Cr(III) enters cells by passive diffusion or phagocytosis of precipitates resulting in much lower uptake. Human and animal studies show that chromium is widely distributed in the body after exposure to Cr(VI), with liver, kidney, spleen, and bone having higher concentrations than other tissues (NTP, 2008).

Current analytical procedures cannot differentiate between the oxidation states of chromium in biological tissues so total chromium content is measured in tissues (NTP, 2008). Hexavalent chromium is able to penetrate the cell membrane of red blood cells (RBC), apparently due to its uptake through anion channels in the plasma membrane. The structures responsible for the uptake of Cr(VI) into RBC are present in other cells; therefore, other cells would be expected to readily take up Cr(VI). Oral, intra-tracheal, intravenous (i.v.), or i.p. administration of Cr(VI) results in increased chromium levels in a number of tissues, while little uptake occurs following the administration of Cr(III) (Mackenzie et al., 1958; Baetjer et al., 1959; Yamaguchi et al., 1983; Wiegand et al., 1984; Edel and Sabbioni, 1985; NTP, 2007).

Chromium has been shown to be absorbed by humans during occupational inhalation exposures. Factors influencing the extent of absorption of inhaled chromium include: particle size, oxidation state, and solubility; activity of alveolar macrophages; and the interactions of chromium with biomolecules in the lung. Reduction of Cr(VI) to Cr(III) occurs in lung tissue (U.S. EPA, 1998).

Distribution

In several studies where Cr(VI) was administered to rats and mice in drinking water, chromium was found to cross the placenta and reach fetal tissues (Trivedi et al., 1989; Saxena et al., 1990a; Junaid et al., 1995; Kanojia et al., 1996; Junaid et al., 1996a; Junaid et al., 1996b; Kanojia et al., 1998; Elsaieed and Nada, 2002). Oral, intra-tracheal, i.v., or i.p. administration of Cr(VI) results in increased chromium levels in a number of tissues,

while little uptake occurs following the administration of Cr(III) (NTP, 2008). The widespread distribution of chromium into tissues following Cr(VI) administration by inhalation, intra-tracheal installation, subcutaneous (sc) injection, i.p. injection and ingestion indicates that although reduction is likely to be occurring in the blood, it does not occur at a fast enough rate to prevent Cr(VI) from reaching and being taken up by tissues. Hexavalent chromium administered by the oral route resulted in elevated chromium levels in the liver, kidney, and spleen but only modestly elevated in RBC (NTP, 2008). Significantly increased tissue concentrations of total chromium were observed in the erythrocytes, liver, kidney, forestomach, and glandular stomach of exposed rats and mice compared to controls indicating that additional Cr(VI) absorption occurs in these tissues, where it appears to accumulate with exposure concentration and time (NTP, 2008).

The half-life of chromium in various tissues (other than plasma) of rats administered Cr(VI) exceeded 20 days (Weber, 1983). The prolonged urinary half-life following oral administration of Cr(VI) (39 hours) compared to Cr(III) (10 hours) suggests that Cr(VI) but not Cr(III) is being taken up and then eluted from cells (NTP, 2008). Given little increase in RBC chromium levels following oral administration of Cr(VI), the liver is likely to be an important site of cellular uptake of Cr(VI) (NTP, 2008).

Reduction of Cr(VI) to Cr(III)

It has been suggested that both enzymatic and nonenzymatic pathways may be involved in chromium reduction, although at normal physiological conditions, nonenzymatic reduction is believed to dominate. The primary reductants of Cr(VI) are ascorbic acid, glutathione, and cysteine, with ascorbic acid being the main reductant (NTP, 2008).

Excretion

Ingested chromium is excreted primarily in the feces because of its poor absorption. Absorbed chromium appears to be primarily excreted in the urine (NTP, 2008).

B.4. Non-DART toxicities

B.4.1. Human studies

Oral exposures of humans to Cr(VI) have occurred through contamination of well water or other accidental poisoning (U.S. EPA, 1998). Reported effects included: mouth ulcers, diarrhea, abdominal pain, indigestion, vomiting, leukocytosis, presence of immature neutrophils, metabolic acidosis, acute tubular necrosis, kidney failure, and death. Analyses of cancer mortality data from a Chinese village exposed to contaminated well water found suggestive evidence of increased stomach cancer risk (Beaumont et al., 2008).

Inhalation exposures of humans to Cr(VI) can occur to workers in industries such as chromate production, chrome plating, ferrochromium production, gold mining, leather tanning, and chrome alloy production (U.S. EPA, 1998). Several epidemiological studies have focused on exposures occurring from stainless steel welding. A number of epidemiological studies, conducted in several countries, have found correlations between occupational exposures to chromium and lung cancer. After systematic reviews of these studies and other relevant data, a number of national and international bodies have identified Cr(VI) as a known human carcinogen (IARC, 1990; U.S. EPA, 1998; NTP, 2005; OEHHA, 2008). Hexavalent chromium is also a recognized respiratory toxicant in humans, causing nasal atrophy, ulcerations, septal perforations, pulmonary function changes, and other respiratory effects (ATSDR, 2000; OEHHA, 2000). Costa (1997) notes that because Cr(VI) forms at physiological pH oxyanions, quite similar to sulfate and phosphate anions, it is able to penetrate cells throughout the body. Besides the respiratory tract, target organs for toxicity observed after human exposure to Cr(VI) include the gastrointestinal system (e.g., duodenal ulcers), eyes and conjunctiva, kidney, liver and hematopoietic system (OEHHA, 2000). Harmful effects are speculated to be related to the reduction of Cr(VI) to Cr(III) intracellularly when it crosses the cell membrane and forms complexes with intracellular macromolecules (OEHHA, 2000).

B.4.2. Animal studies

Reviews of the literature on chronic and subchronic Cr(VI) exposures of experimental animals by both the oral and inhalation routes (U.S. EPA, 1998; ATSDR, 2000; OEHHA, 2000) include some reports of effects similar to those observed in human occupationally exposed to Cr(VI).

Recently, the National Toxicology Program (NTP, 2008) completed drinking water toxicology and carcinogenicity studies in mice and rats of Cr(VI) (as sodium dichromate dehydrate). These studies were the consequence of a nomination by the California Environmental Protection Agency (Cal EPA), California Department of Health Services (CDHS) and California Congressional Delegation because of lack of adequate data for addressing drinking water exposure to Cr(VI). Cellular infiltration in the liver, small intestine, and pancreatic and mesenteric lymph nodes was observed in rats and mice and diffuse epithelial hyperplasia in the small intestine was observed in male and female mice. All mice Cr(VI) treatment groups were affected with epithelial hyperplasia; the corresponding non-cancer LOAEL was 1.1 mg/kg day sodium dichromate dehydrate or 0.4 mg/kg Cr(VI). The LOAEL for liver lesions in rats (histiocytic cellular infiltration, chronic inflammation, fatty change (females), basophilic focus (males), and clear cell focus (females)) corresponds to 0.8 and 0.9 mg Cr(VI)/kg-d, for male and females respectively, and the NOAEL to 0.2 mg/kg-d.

In addition, the NTP found clear evidence of carcinogenic activity in rats and mice of both sexes, with the small intestine as the site for mice and the oral cavity as the site for rats. Costa and Klein (2006) have found skin tumors in mice resulting from Cr(VI) in drinking water in combination with solar ultraviolet radiation.

The NTP (2007) also recently published oral short term (3 month) studies of Cr(VI) (as sodium dichromate dehydrate). A microcytic hypochromic anemia was seen in all Cr(VI) rat groups, with the LOAEL (lowest dose tested) corresponding to 1.7 mg Cr(VI)/kg-d. A less severe form of anemia was also seen in mice. Also findings of cellular infiltration and hyperplasia were similar to that observed in the chronic studies.

Chronic inhalation studies have exposed lab animals to a variety of Cr(VI) compounds by methods including breathing dust or aerosols, intratracheal installation, intrabronchial implantation, and intrapleural implantation. Results have included lung and nasal carcinogenicity, as well as other toxic effects on lung tissues. In 1990, IARC reviewed these studies and concluded there was sufficient evidence of carcinogenicity in animals. Subchronic inhalation studies of Cr(VI) have also found the lung, as well as the immune system, to be target tissues. The IARC (1990) notes that "Lead chromate and derived pigments have also been tested in rats by subcutaneous and intramuscular injection, producing malignant tumours at the site of injection and, in one study, renal carcinomas."

There have been a number of reviews of the genotoxicity data for Cr(VI). The NTP (2008) recent review summarized the evidence as follows: "Hexavalent chromium is genotoxic in a number of in vitro and in vivo test systems, although responses are somewhat variable depending on protocol details and the type of chromium salt that is assayed. Overall, the data clearly indicate that in appropriate test systems, Cr(VI) exposure results in increased frequencies of gene mutations and chromosomal alterations."

C. DEVELOPMENTAL TOXICITY

C.1. Human developmental toxicity studies

One case-control study of drinking water contamination and one epidemiological study of ecological design addressed developmental toxicity.

Aschengrau et al. (1993). Quality of community drinking water and the occurrence of late adverse pregnancy outcomes.

Aschengrau et al. (1993) published the results of a case-control study of late adverse pregnancy outcomes and drinking water quality in Boston, Massachusetts. The cases were diagnosed at a single hospital in Boston in the time period 1977–1980.
The women were chosen from a previous study examining behavioral risk factors and adverse pregnancy outcomes. There were 14,130 obstetric patients, representing about 82.5% of the deliveries at the hospital during the study years. The cases were selected from this group and retaining women with medical record data (86.6%) resulted in 1039 cases of congenital anomalies, 77 stillbirth cases, 55 neonatal deaths and 1179 controls (chosen randomly for roughly a 1:1 ratio of cases to controls). Since the 1960s, the Massachusetts Department of Environmental Protection routinely collected representative

water samples from all the cities in the state with public supplies and, since 1977, has performed routine chemical and metal samples. Each woman was matched to the sample in her city's water supply that was obtained closest to her conception date. The difference in sample date and conception date ranges from 0 days to 4.1 years, or 2.6 years for chemicals and metals, respectively, with median of 3.3 months to 5.9 months for the chemical and metal analyses, respectively.

The residential histories of the cases and controls were geo-linked to routine drinking water quality data for a wide variety of metals (e.g., arsenic, cadmium, chromium, lead, mercury, silver, etc.) and other parameters of drinking water quality. The median levels of chromium were below the detection limit, with zero concentrations at the 90th percentile, and 0.0110 mg/ml at the highest level detected. It is unclear if the trace amount of chromium detected by the authors was Cr(III) or Cr(VI); however, as discussed in Section B.2, Cr(VI) can be a contaminant of drinking water. Crude and adjusted OR were calculated using multiple logistic regression for each outcome (all congenital anomalies, include a few severity subgroups and organ systems, still births and neonatal deaths), with categorical metal (not detectable, detectable or 95th percentile) and chemical (neutral, acidic or alkaline, or tertiles) exposure variables. The confounding variables included in the adjusted estimates were dichotomous variables for maternal race, age, payment method, and history of congenital anomaly. Average number of alcoholic drinks per week during first trimester (0, 1–2, ≥3) and water source (surface, ground, mixed) were also included.

The authors found no significant association of chromium levels in the drinking water with all congenital anomalies (adjusted OR=0.8), all stillbirths (adjusted OR=1.2) or all neonatal deaths (crude OR=0.7; no adjusted OR was presented). When congenital anomalies were divided into seven organ system groups, chromium again was not significantly associated with risk, although the OR for musculoskeletal defects was decreased with borderline significance (OR=0.4, 95% CI 0.2–1.0). For chromium, the risk for still births was elevated in both the crude and adjusted estimates, but neither was statistically significant. Significant associations were present for other metals (arsenic, lead, mercury, and selenium for the integument system and sliver for ear, face and neck). Water treatment type (chlorination) was also significantly associated with still births.

There are several limitations to this study. First, the classifications of chemical and metal levels in the woman's water supply are unlikely to be representative of her actual exposure since the samples were taken from a public building and not her house. The amount of water consumed by the mother is not included in the study and neither is the amount of bottled water or her diet exposures. Second, the time delay between conception and water sampling also provides an uncertainty in the mother's exposure, especially when the difference falls in the upper end of the range. Time trends of the contamination values were not discussed. Third, other contaminates not tested (organics) and external exposures such as air and occupational are also not included. Fourth, the chemical and metal variables were categorical and did not have much specificity. While there were detectable levels for chromium (and other metals and chemicals), there was not much variability between the study subjects. The authors note that if 10- to 100- fold differences present in metals such as arsenic, barium, lead, mercury and selenium were seen in chromium (and other variables), then the effects would be more pronounced.

An important aspect of the study is that the original purpose of selecting the cases and controls was to examine the role of behavioral risk factors (not water quality) on late adverse pregnancy outcome. The linkage to water quality data was a secondary analysis that was hypothesis generating (rather than testing) in its purpose.

Eizaguirre-Garcia et al. (2000). Congenital anomalies in Glasgow between 1982 and 1989 and chromium waste.

Eizaguirre-Garcia *et al*. (2000) performed a descriptive geographical study around an old chromium-processing factory in Glasgow, Scotland. Substantial chromium soil contamination was found in March of 1991. Areas of widespread contamination had total chromium in soil measured to be 100–200 parts per million (ppm), and at the old factory, mean total chromium was 8164 ppm. The authors assumed that those living close to the factory site had an increase risk of chromium exposure.

To examine the effect of congenital anomalies, the authors calculated relative risks (RR) based on radial distance from the site. The first circle was 2 km in radius centered on the factory site and was the baseline. The remaining eight circles were 1 km wide, enveloping a total radius of 10 km. Enumeration Districts (EDs), typically with 350–500 people each, were used to ascertain population demographic data. The population in each of the rings was obtained by summing the population of the EDs whose centroid lay in each of ring. About 16% (441 out of 2780) of the EDs were not included in the outer rings because of the "irregular shape of its borders." Carstairs deprivation categories were used (1 the lowest, 7 the highest) as indicators for socioeconomic status. A deprivation category value was unavailable for 72 of the EDs. The Greater Glasgow Health Board's Health Information unit provided the total number of cases and births for eight years, between 1982 and 1989. There were 2937 cases, but 72 had to be removed due to coding errors (69 cases) and missing data (3 cases). A total of 81,057 births make up the remaining population. For the analyses that include the deprivation category variable, an additional 19 case and 492 births were removed from the study because their ED did not have deprivation category value.

The authors calculated crude and adjusted relative risks using Poisson regression based on distance from the center of the factory site. When distance was the only variable included in the model the RR was the highest in the 2–3 km ring (1.52; 95 CI1.24–1.85) and 3–4 km ring (1.40; 95% CI 1.15–1.70). The 4–5 km and 5–6 km rings did not have significant RR, but the 6–7 km (1.23) and 7–8 km (1.30) rings were significantly elevated. When deprivation category was included in the model (without distance) all the RR were significant. With both variables in the model, the RR remains peaked at the 2–3 km distance (1.50). The authors conclude that the central 2 km circle had a lower risk of congenital anomalies than several of the outer rings, and any level of deprivation but the lowest resulted in elevated risk. Thus, they concluded that teratogenicity was not induced by chromium exposure.

The study has several limitations, a few of which are highlighted here. First, there was neither control for maternal age, a major determinant of congenital abnormality, nor for alcohol consumption. This may partially explain the reason for the higher RR values in

areas with deprivation scores indicative of higher socioeconomic status. Second, the study design was only able detect effects based on the author's arbitrary geographical rings. The authors note that the two highest areas of chromium concentration are at the factory site and then 2–3 km *southeast* of the factory (e.g., in an area associated with a higher relative risk). Without spatial chromium levels it is unknown if the geographic circles are coincident with higher chromium levels, except in the central circle. A cluster analysis may be more important than radial distance. The site of the second highest chromium soil contamination values was not discussed further and was not formally included in the evaluation of the relationship between chromium exposure and malformation. Third, other potential co-pollutants or other confounders are not addressed or discussed. Fourth, the type of congenital effect was not taken into account, and all congenital malformations were lumped together.

C.2. Animal developmental toxicity studies

Available data on the potential developmental toxicity of Cr(VI) in experimental animals includes studies with gestational exposure alone and others with pre-conceptual exposure alone. The gestational exposure studies are oral drinking water studies, one in rats (Elsaieed and Nada, 2002), and four in mice (Trivedi et al., 1989; Junaid et al., 1995; Junaid et al., 1996a; De Flora et al., 2006). One of these studies (De Flora et al., 2006) also exposed mice via i.p. injection.

For pre-conceptual exposures, data are provided by studies of female reproductive toxicity in which adverse effects on the developing offspring were reported (Kanojia et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Kanojia et al., 1998).

C.2.1. Gestational exposure

Rat, oral (drinking water)

Elsaieed and Nada. (2002). Teratogenicity of hexavalent chromium in rats and the beneficial role of ginseng.

Timed-pregnant (plug day = day 0) Wistar rats were randomly divided into four groups of 10, and treated as follows: 1) untreated control, 2) 50 ppm Cr(VI) as potassium chromate (K_2CrO_4) in drinking water on gestation days 6–15, 3) same as #2, with the addition of 20 mg ginseng /kg body weight, daily, by gavage, 4) ginseng only, given as for #3. The authors note that a concentration of 50 ppm Cr(VI) was chosen as preliminary experiments had found complete implantation loss with a concentration of 100 ppm.

No maternal mortality was reported, and there were no clinical signs of toxicity. No significant alterations in food or water intake were noted among groups. Maternal gestational weight gain was significantly lower in animals given 50 ppm Cr(VI) as compared to controls ($p<0.05$). The number of corpora lutea per litter was not affected by treatment. Pre- and post-implantation loss, resorption frequency, and the frequency of dead fetuses per litter were all significantly increased with Cr(VI) exposure ($p<0.05$ for

all endpoints). The number of live fetuses/litter was decreased from 6.8 in controls to 1.5 for the Cr(VI)-exposed group ($p<0.05$ for all endpoints). Fetal weights were significantly decreased with Cr(VI) exposure ($p<0.05$). The frequencies of visceral and skeletal anomalies were significantly increased in Cr(VI)-exposed litters ($p<0.05$). In particular, these anomalies consisted of renal pelvis dilatation and incomplete ossification of skull bones.

Table C1. Developmental toxicity in Wistar rats – drinking water study by Elsaieed and Nada (2002)

Parameter	Control	50 ppm chromium as potassium chromate
Maternal wt gain (g)	23.6 $\pm$ 1.3	14.2 $\pm$ 1.7*
Pre-implantation loss/litter	0	2.1 $\pm$ 0.36*
Post-implantation loss/litter	0	1.5 $\pm$ 0.34*
Resorptions/litter	0	1.2 $\pm$ 0.13*
Dead fetuses/litter	0.1 $\pm$ 0.099	1.2 $\pm$ 0.24*
Live fetuses/litter	6.8 $\pm$ 0.44	1.5 $\pm$ 0.29*
Mean fetal wt	3.9 $\pm$ 0.42	2.6 $\pm$ 0.23*
No. visceral anomalies/litter	0	2.1 $\pm$ 0.39*
No. skeletal anomalies/litter	0	1.0 $\pm$ 0.34*

*$p<0.05$

Histopathological observations revealed chromium-induced lesions in placental tissues. Significant increases in chromium content were found in the maternal blood, fetal tissues, and placentas of treated animals ($p<0.05$ for each endpoint). The fetal/placental chromium content ratio was also significantly increased in chromium-treated animals ($p<0.05$).

Ginseng alone had no effect on any of the maternal or fetal endpoints measured. Ginseng given along with chromium significantly ($p<0.05$ in each case) ameliorated the adverse effects of chromium for most endpoints, as well as lowering the amount of chromium found in the maternal blood, placentas, fetuses, and fetal/placental ratio (not significant for placentas, $p<0.05$ for other endpoints).

Mouse, oral (drinking water)

De Flora et al. (2006). Oral chromium (VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses.

As part of a series of experiments designed to look at the cancer-causing potential of oral exposure to Cr(VI), timed-pregnant Swiss albino mice were given sodium dichromate dihydrate ($Na_2Cr_2O_7 \cdot 2H_2O$) or potassium dichromate ($K_2Cr_2O_7$). Five dams were kept as untreated controls. Two groups of five dams were give sodium dichromate dihydrate in their drinking water to concentrations of 5 or 10 mg Cr(VI)/L throughout gestation. One group of five dams was given potassium dichromate in their drinking water to a concentration of 10 mg Cr(VI)/L throughout gestation. Two additional groups of five dams each were given a single i.p. injection of sodium dichromate dihydrate or potassium dichromate, at a dose of 50 mg/kg body weight, in a total volume of 0.2 ml of sterile distilled water. All dams were sacrificed on gestation day 18 (plug day designated day 1), and the left femur removed for analysis of bone marrow cells. Litter sizes and fetal weights were determined. Liver and blood samples were collected from fetuses.

Neither litter size nor fetal weights were affected by treatment. Neither sodium dichromate dihydrate nor potassium dichromate by the oral route had any significant effect on the frequency of micronucleated polychromatic erythrocytes (MN PCE) in maternal bone marrow or fetal liver or peripheral blood. Neither compound by the oral route had any significant effect on the ratio of PCE to normochromatic erythrocytes (NCE) in maternal bone marrow or fetal liver or peripheral blood. On the other hand, i.p. injection of either compound was associated with significant increases in the frequencies of MN PCE for maternal bone marrow, fetal liver, and fetal peripheral blood ($p<0.001$ compared to controls in all cases). The PCE/NCE ratios were not affected by intraperitoneal injection of Cr(VI) compounds.

Junaid et al. (1996a). Embryotoxicity of orally administered chromium in mice: exposure during the period of organogenesis.

Timed-pregnant Swiss albino mice (plug day = day 0) were divided into four groups of 10. On gestation days 6–14, animals were given potassium dichromate in drinking water to Cr(VI) concentrations of 0, 250, 500, or 750 ppm. Caesarian sections for examination of uterine contents were performed on gestation day 19.

No maternal mortality occurred during the experimental period, and no clinical signs of toxicity were observed. Data on water intake allowed calculation of actual doses, which worked out to averages of 0, 2.00, 3.75, or 5.47 mg/mouse/day, for the 0, 250, 500, and 750 ppm groups, respectively. Maternal weight gain was significantly reduced for dams in the 500 and 750 ppm groups ($p<0.05$ at both concentrations) in a dose-related manner.

Numbers of corpora lutea, placental weights, and fetal CRL were unaffected by treatment. In the low-concentration group (250 ppm or 2.0 mg/mouse/day), the only significant effect observed was an increase in the number of resorption sites per litter ($p<0.05$). The number of resorption sites per litter was significantly increased in all three treated groups in a concentration-dependent manner. Dose groups showed significant differences from each other as well as from controls ($p<0.05$ in all cases). Both the total number of fetuses (live and dead) per litter and fetal weight were significantly ($p<0.05$)

reduced in the 500 and 750 ppm groups (3.75 and 5.47 mg/mouse/day, respectively). The number of live fetuses per litter was not analyzed separately. Post-implantation loss was significantly increased over controls in the mid and high-concentration/dose groups ($p<0.05$); greater loss was seen at 750 ppm than at 500 ppm ($p<0.05$).

Table C2. Developmental toxicity in Swiss mice – drinking water study by Junaid et al. (1996a)

Parameter/Cr(VI) concentration[a]	Control	250 ppm	500 ppm	750 ppm
Maternal wt gain	15.57 ± 0.20	15.21 ± 0.31	14.29 ± 0.54*	11.79 ± 0.49*
Total fetuses/litter	8.8 ± 0.29	8.20 ± 0.20	7.0 ± 0.36*	7.20 ± 0.24*
Fetal wt	1.31 ± 0.41	1.27 ± 0.03	1.14 ± 0.03*	1.06 ± 0.029*
Resorptions/litter	0.30 ± 0.21	1.00 ± 0.21*	1.70 ± 0.3*	2.30 ± 0.273*
Post-implantation loss %	4.32 ± 2.34	10.60 ± 2.11	21.93 ± 3.96*	34.60 ± 2.54*

* $p < 0.05$
[a] As potassium dichromate

No significant increases in frequencies of gross external abnormalities were seen at 0, 250, or 500 ppm chromium. Fetuses from the 750 ppm group showed significant ($p<0.05$ in both cases) increases over controls in the frequencies of "drooping wrist" and "subdermal hemorrhagic patches." No gross visceral abnormalities were observed in any group.

The frequency of "reduced caudal ossification" was significantly increased in both the 500 and 750 ppm groups ($p<0.05$ for both concentrations). While there appeared to be a concentration-related increase in this endpoint, the difference between groups was not significant. The 750 ppm group also showed significant increases over controls in the frequencies of "reduced nasal ossification," "reduced frontal ossification," "reduced parietal ossification," "reduced interparietal ossification," and "reduced tarsals ossification" ($p<0.05$ for each endpoint).

Samples of maternal cardiac blood taken at the time of sacrifice, and one fetus per litter were retained with their placentas for determination of chromium levels. The chromium content of maternal blood increased significantly with increasing concentration of chromium in the drinking water. All differences among groups were statistically significant at $p<0.05$. The chromium contents of fetuses and placentas showed the same pattern, with all differences among groups significant at $p<0.05$.

Junaid et al. (1995). Chromium fetotoxicity in mice during late pregnancy.

Timed-pregnant Swiss albino mice were divided into four groups of 10 each. The animals were given Cr(VI) (as potassium dichromate) in drinking water at concentrations

of 0, 250, 500, or 750 ppm from gestation day 14 through 19. On gestation day 19, the animals were sacrificed for caesarian section and examination of uterine contents.

No behavioral or clinical signs of toxicity were observed. Gestational weight gain was not affected in dams of the 250 ppm group, but was significantly decreased at 500 and 750 ppm Cr(VI) ($p<0.001$), in a concentration-dependent manner. There were no significant differences among groups for the numbers of corpora lutea, fetuses/litter (unclear from the paper whether this is live or live plus dead), dead fetuses, or resorption sites. Postimplantation loss was significantly increased over controls in the 500 and 750 ppm groups ($p<0.001$), in a concentration-dependent manner. Placental weights were significantly *increased* at 500 and 750 ppm ($p<0.001$), in a concentration-dependent manner. Both fetal weights and CRL decreased with increasing Cr(VI) concentration in all treated groups. For CRL, statistical significance in the 250 ppm group reached the $p<0.05$ level, while changes in the other two groups were significant at $p<0.001$. For fetal weight, statistical significance in the 250 ppm group reached the $p<0.01$ level, while changes in the other two groups were significant at $p<0.001$.

Table C3. Developmental toxicity in Swiss mice – drinking water study by Junaid et al. (1995)

Parameter/Cr(VI) concentration[a]	Control	250 ppm	500 ppm	750 ppm
Maternal wt gain (g)	15.37 ± 0.34	15.37 ± 0.31	13.67 ± 0.20***	11.33 ± 0.38***
Fetuses/litter	8.3 ± 0.29	8.8 ± 0.29	8.3 ± 0.33	8.4 ± 0.26
No. dead fetuses (No litters with dead fetuses)	0	1 (1)	13 (6)	20 (10)
Fetal wt (g)	1.59 ± 0.06	1.31 ± 0.04**	1.00 ± 0.03***	0.84 ± 0.03***
Placental wt (g)	0.152 ± 0.006	0.155 ± 0.007	0.19 ± 0.003***	0.203 ± 0.006***
Crown-rump length (CRL) (cm)	2.73 ± 0.02	2.62 ± 0.03*	2.33 ± 0.02***	1.94 ± 0.03***
Resorptions/litter	0	0.30 ± 0.21	0.40 ± 0.16	0.40 ± 0.16
Post-implantation loss/litter	0	3.51 ± 2.30	18.8 ± 3.85***	27.30 ± 2.02***

*p < 0.05, **p < 0.01, ***p < 0.001

[a] As potassium dichromate

No significant visceral anomalies were observed in any of the groups. Gross external anomalies observed at 750 ppm at statistically significant frequencies included: "drooping wrist" ($p<0.01$), "sub-dermal hemorrhagic patches" ($p<0.01$), "kinky tail" ($p<0.05$), and "short tail" ($p<0.01$). In the 500 ppm group, "drooping wrist" was also observed at a significantly higher frequency than among controls ($p<0.05$). Significantly increased frequencies of "reduced caudal ossification" were observed in all treated

groups (P<0.01 or P<0.001). No other skeletal anomalies were observed among controls or the 250 ppm group. "Reduced tarsal ossification" was observed at significant frequencies in both the 500 and 750 ppm groups (p<0.001 for both groups). Other skeletal anomalies observed at statistically-significant frequencies (p<0.001) in the 750 ppm group included: "reduced nasal ossification," "reduced parietal ossification," "reduced inter-parietal ossification," "reduced carpal ossification," and "reduced metacarpals ossification."

Chromium levels in maternal blood, placenta, and fetuses significantly increased with increasing Cr(VI) intake (p<0.001). Chromium appeared to accumulate in placenta, with a slower rate of transfer from placenta to fetus than from maternal blood to placenta.

Trivedi et al. (1989). Embryotoxicity and fetotoxicity of orally administered hexavalent chromium in mice.

Groups of ten timed-pregnant female ITRC-bred albino mice were given Cr(VI) in drinking water at concentrations of 0, 250, 500, or 1000 ppm as potassium dichromate. Treatment began on gestation day 0 (the day a seminal plug was detected) until gestation day 18. Sacrifice for examination of uterine contents was performed on gestation day 19.

Table C4. Consumption of drinking water containing potassium dichromate dichromate in the study by Trivedi et al. (1989)

Concentration	Water consumed: ml/mouse-day	Cr(VI) consumed: mg/mouse-day
controls	9.03 ± 0.42	0
250 ppm	7.05 ± 0.97	1.76 ± 0.24
500 ppm	7.20 ± 1.30	3.6 ± 0.65
1000 ppm	7.03 ± 0.28	7.03 ± 0.28

There was no maternal mortality observed during the study period, and no clinical or behavioral signs of toxicity were observed for dams in any group. At the highest concentration of potassium dichromate (1000 ppm), no live litters or implantation sites were found. These animals had significantly lower body weight gain than controls over the study period (p<0.001). Weight gain was also significantly decreased in the 500 ppm group (p<0.01).

As there were no litters in the 1000 ppm group, there are fetal data only for the 250 and 500 ppm groups. The numbers of corpora lutea, placental weights, and sex ratio were not affected by treatment. Litter size was significantly lower than controls in the 500 ppm group (p<0.01). Fetal weights and CRL were significantly decreased at both 250 and 500 ppm (p<0.001 for both endpoints in both groups). Resorption frequency was significantly increased at both 250 (p<0.001) and 500 ppm (p<0.01). Preimplantation loss was significantly greater than controls for the 500 ppm group (p<0.001). Postimplantation loss was significantly increased in the 250 and 500 ppm groups (p<0.001 in both groups).

Table C5. Embryotoxic and fetotoxic effects in mice – drinking water study by Trivedi et al. (1989)

Cr(VI) Exposure group^	**Control**	**250 ppm**	**500 ppm**	**1000 ppm**
Number of litters (number of females)	10 (10)	10 (13)	10 (12)	0 (10)
Body weight gain (g)	16.43±2.10	13.5±1.94	13.0±0.65[a]*	-1.19±2.8[abc]***
Litter size	6.3±0.61	3.8±0.73	3.5±0.57 [a]*	No implantation
Corpora lutea	6.6±0.72	5.0±0.71	8.0±0.54	No implantation
Placental weight (mg)	134.0±5.00	123.0±4.37	126.0±4.30	No implantation
Sex ratio (M/F)	2.40/4.00	2.25/1.50	1.50/2.00	No implantation
Fetal weight (g)	1.95±0.08	1.32±0.05 [a]***	1.09±0.03 [a]*** [b]**	No implantation
Crown-rump length (CRL) (cm)	2.87±0.12	2.38±0.08 [a]***	2.05±0.12 [a]*** [b]**	No implantation
Resorption frequency (%)	1 (10.0)	9 (32.69) [a]***	7 (51.70) [a]**	No implantation
Preimplantation loss (%) (number of incidences)	3.60±2.60 (2)	7.88±3.36 (3)	26.19±1.54 [a]*** [b]** (6)	No implantation
Postimplantation loss (%) (number of incidences)	1.66±1.25 (1)	26.48±1.46 [a]*** (3)	88.09±2.12 [ab]*** (7)	No implantation

(Adapted from Trivedi et al., 1989)
^as potassium dichromate
The values represent means ±SEM of 10 mice in each group. The significance of the difference among various groups was evaluated by applying one-way ANOVA, except for incidence of resorptions, where Fisher Exact Test was used for comparison.
Comparison between two groups:
[a] = vs control, [b] = vs 250 ppm, [c] = vs 500 ppm.
*p < 0.05, **p < 0.01, ***p < 0.001.

Increases in the frequency of kinked tail and subdermal hemorrhages were statistically significant in the 500 ppm group ($p<0.05$). The frequencies of several ossification insufficiencies, as well as in reduced ribs, were seen at 500 ppm ($p<0.05$ or $p<0.001$). Reduced cranial ossification was also significant for the 250 ppm group ($p<0.01$). No internal soft-tissue anomalies were observed.

Table C6. Incidences of gross and skeletal abnormalities in mice – drinking water study by Trivedi et al. (1989)

Cr(VI) Exposure group^	Control	250 ppm	500 ppm
Gross abnormalities pups/litter (%)[a]			
Number of pups/litter observed	30/10	30/6	14/7
Kinked tail	–	5/4 (16.67)	3/3 (21.4)*
Subdermal hemorrhagic patches/streaks	2/1 (6.66)	12/4 (40.0)	7/5 (50.0)*
Enlarged gaps between fingers	–	2/2 (6.67)	2/2 (14.3)
Drooping wrist	–	3/3 (10)	2/2 (14.3)
Skeletal abnormalities pups/litter (%)			
Number of pups/litter observed	24/6	20/5	10/5
Reduced cranial ossification	–	8/4 (40)**	6/5 (60)***
Reduced forelimb ossification (phalanges)	–	–	8/5 (80)***
Reduced hind limb ossification (phalanges)	1/1 (4.2)	–	6/4 (60)***
Reduced number of ribs	–	2/2 (10)	3/3 (30)*
Reduced sternebrae ossification	–	–	5/4 (50)***
Reduced ossification of thoracic vertebrae	–	2/2 (10)	3/3 (30)*
Reduced ossification of caudal vertebrae	1/1 (4.2)	–	7/5 (70)***

(Adapted from Trivedi et al., 1989)
^as potassium dichromate
Statistical significance evaluated by Fischer Exact Test. Significance level; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Group administered 1000 ppm Cr (VI) did not show any implantation
[a]Percentage in parentheses calculated by the total number of pups observed.

Statistically significant increases in chromium levels were found in maternal blood of the 1000 ppm group ($p<0.001$), placentas at both 250 and 500 ppm ($p<0.001$), and fetuses of the 500 ppm group ($p<0.01$).

C.2.2. Dam exposed prior to mating

Rat, oral (drinking water)

Kanojia, et al. (1998). Embryo and fetotoxicity of hexavalent chromium: a long-term study.

Female rats of the "Druckery" strain were given potassium dichromate in drinking water to Cr(VI) concentrations of 0, 250, 500, or 750 ppm. Treatment was begun when animals

were 50 days of age, and continued for a period of three months. At the end of the treatment period, each female was caged for mating with a single, untreated male. The presence of sperm in a daily vaginal smear was considered to represent day 0 of pregnancy. Ten dams from each group were sacrificed for Caesarian section on gestation day 19.

Details of female reproductive effects are described in section D.2. "Animal female reproductive toxicity studies," below.

Maternal body weight at the end of gestation and maternal weight gain during gestation decreased with increasing concentration of Cr(VI); the changes reached statistical significance ($p<0.05$) for both endpoints at the two highest concentrations. The numbers of corpora lutea, implantations, and live fetuses per litter all decreased with increasing Cr(VI) concentration, with differences among groups reaching statistical significance ($p<0.05$) at 500 and 750 ppm. Resorption frequency increased with increasing concentration of Cr(VI), reaching statistical significance ($p<0.05$) at 500 and 750 ppm. The frequencies of both pre- and post-implantation loss were significantly increased relative to controls in all treated groups ($p<0.05$), in a concentration-dependent manner. Fetal weight decreased with increasing Cr(VI) concentration, showing statistically significant differences from controls ($p<0.05$) at all concentrations. Mean placental weights and fetal CRL both decreased with increasing concentration, and both reached statistical significance in the 500 and 750 ppm groups ($p<0.05$).

Table C7. Developmental toxicity in Druckrey female – dams exposed via drinking water prior to mating Kanojia, et al. (1998)

Parameter/Cr(VI) concentration[a]	**Control**	**250 ppm**	**500 ppm**	**750 ppm**
Maternal wt gain (g)	69.16 ± 3.66	61.66 ± 4.23	57.50 ± 2.82*	54.16 ± 3.11*
Live fetuses/litter	9.30 ± 0.92	7.30 ± 0.53	5.45 ± 0.42*	4.16 ± 0.31*
Fetal wt (g)	4.36 ± 0.28	3.44 ± 0.22*	3.07 ± 0.16*	2.75 ± 0.22*
Placental wt (g)	0.72 ± 0.06	0.67 ± 0.07	0.52 ± 0.05*	0.37 ± 0.08*
Resorptions/litter	0.53 ± 0.25	1.02 ± 0.30	1.41 ± 0.37*	1.67 ± 0.34*
Post-implantation loss %	5.39 ± 0.35	12.25 ± 0.63*	20.25 ± 0.54*	22.80 ± 0.91*

*$p < 0.05$
[a] As potassium dichromate

No gross external or visceral anomalies were observed among control fetuses. The only skeletal anomaly observed in controls was "reduced caudal ossification," which was present in five fetuses from three litters. No gross visceral anomalies were seen in any of the treated groups. Externally, significantly increased frequencies ($p<0.05$) of "drooping wrist" and "subdermal hemorrhagic patches" were observed in all treated groups. Significantly increased frequencies ($p<0.05$) of "kinky tail" and "short tail" were observed at the two higher concentrations. Skeletal evaluations revealed increased

frequencies of “reduced caudal ossification” in all treated groups (significant differences among all groups at $p<0.05$), which occurred in a concentration dependent manner.

Chromium concentrations in maternal blood, placenta, and fetal tissues increased significantly with increasing Cr(VI) intake ($p<0.05$ among all groups). When chromium content was considered as a ratio of placenta to maternal blood or fetus to placenta, all treated groups were significantly increased relative to controls ($p<0.05$), but there was no significant additional increase with increasing intake.

Kanojia et al. (1996). Chromium induced teratogenicity in the female rat.

Adult Swiss albino female rats were given potassium dichromate in drinking water at concentrations selected to provide 0, 250, 500 or 750 ppm Cr(VI). Based on water intake data collected during the study, these concentrations were determined to have provided doses of 0, 6.44, 12.2, and 15.28 mg/kg-day. The animals were treated for 20 days. Once treatment was ended, the females were caged (1:1) with untreated males for mating. Ten pregnant females from each group were sacrificed on gestation day 19 (day 0 = day sperm detected in vaginal smear) for examination of reproductive organs and uterine contents.

Details of female reproductive effects are described in section D.2. “Animal female reproductive toxicity studies,” below.

Relative to controls, maternal weight gain was significantly decreased at all concentrations of Cr(VI), in a concentration-related manner. The numbers of corpora lutea decreased with increasing Cr(VI) concentration, showing statistically significant reductions ($p<0.05$) from controls in the 500 and 750 ppm groups. Implantation frequency was significantly ($p<0.05$) lower than controls for the 500 and 750 ppm groups. The number of live fetuses per litter showed a concentration-dependent decrease (from a mean of 9.11 in controls to 1.21 at 750 ppm). Differences from controls were statistically significant ($p<0.05$) at all Cr(VI) concentrations. Conversely, resorption frequency increased with increasing Cr(VI) concentration; a change that was significantly different from controls ($p<0.05$) at all concentrations tested. Pre-implantation loss was significantly greater ($p<0.05$) in treated than control groups for the 500 and 750 ppm groups. Post-implantation loss showed a striking and significant ($p<0.05$) increase with increasing Cr(VI) concentration, at all concentrations. Placental weights increased with increasing Cr(VI) concentration; the differences were statistically significant ($p< 0.05$) at all concentrations tested.

Table C8. Developmental toxicity in Swiss rats - dams exposed via drinking water prior to mating Kanojia, et al. (1996)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
Maternal wt gain (g)	70.05 ± 5.19	65.02 ± 3.17*	60.92 ± 2.13*	55.5 ± 3.01*
Live fetuses/litter	9.11 ± 0.87	8.29 ± 0.93*	4.12 ± 0.51*	1.21 ± 0.13*
Fetal wt (g)	3.54 ± 0.41	3.46 ± 0.29	3.08 ± 0.37	2.53 ± 0.31
Placental wt (g)	0.67 ± 0.08	0.71 ± 0.09*	0.79 ± 0.19*	0.86 ± 0.12*
Resorptions/litter	0.40 ± 0.24	1.09 ± 0.34*	1.72 ± 0.23*	1.03 ± 0.29*
Post-implantation loss %	4.20 ± 0.41	13.73 ± 1.57*	30.28 ± 4.19*	46.69 ± 5.21*

*$p < 0.05$

[a] As potassium dichromate

Fetal weight and CRL were not significantly affected by maternal pre-gestational exposure to Cr(VI). Gross abnormalities and skeletal anomalies were found at increased frequencies among litters of the 750 ppm group (all significant at $p<0.05$). Specific abnormalities were: sub dermal hemorrhagic patches, kinky tail, short tail, reduced parietal and inter-parietal ossification, and reduced caudal ossification. Reduced caudal ossification was also seen at a significantly increased frequency ($p<0.05$) among litters of the 500 ppm group. No gross visceral abnormalities were seen in fetuses of dams from any of the treated groups.

Chromium levels were measured in maternal blood, placenta, and in fetuses. Fetuses of the 500 and 750 ppm groups showed significant increases in chromium content ($p<0.05$), as compared to controls. Maternal blood and the placentas from all treated groups were significantly higher in chromium content ($p<0.05$) as compared to controls. While the transfer ratio from placenta to fetus did not differ among treated groups, the transfer ratio from the maternal circulation to the placenta was greater for the 750 ppm group than for the other two treated groups.

Mouse, oral (drinking water)

Elbetieha and Al-Hamood. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility.

Adult female Swiss mice were given 0, 2000, or 5000 mg/L (ppm) potassium dichromate for 12 weeks. At the end of treatment, females were housed with untreated, proven fertile males, at a ratio of 3:1, for a period of ten days (presumed to encompass at least two estrous cycles). The animals were sacrificed at one week following the end of mating for examination of their uterine contents.

The numbers of pregnant animals as a fraction of total mated females for the control, 2000, and 5000 ppm groups were as follows: 17/18, 14/15, and 9/11. Relative to

controls, the numbers of implantations per litter were significantly reduced in both the 2000 ppm ($p<0.01$) and 5000 ppm ($p<0.05$) groups. The number of viable fetuses was also significantly reduced at both 2000 ppm ($p<0.05$) and 5000 ppm ($p<0.01$). The number of litters with resorptions was significantly increased at 2000 ppm ($p<0.01$) and 5000 ppm ($p<0.005$).

Table C9. Female reproductive toxicity data: Elbetieha and Al-Hamood, (1997) Treated females mated with untreated males

Parameter	0	2000 mg/L	5000 mg/L
Pregnancy rate (%)	17/18 (94.4)	14/15 (93.3)	9/11 (81.8)
Live fetuses/litter	8.76 ± 1.39[a]	6.55 ± 2.18*	5.88 ± 2.47**
Implantations	9.00 ± 1.36	7.35 ± 1.54**	7.44 ± 1.50*
Number of litters with resorptions (%)	2/18 (11)	8/15 (53)#	7/11 (63)##
Total resorptions	4	37	14

[a] Mean ± S.D.
*p < 0.05; **p < 0.01 (Student's *t* test)
# p < 0.01; ##p < 0.005 (Chi-square test)

The authors suggest that their data are consistent with a chromium-induced disturbance of reproductive endocrine functions, and that the increased resorptions were likely due to modification of the uterine lining prior to arrival of the embryo.

Junaid et al. (1996b). Embryo- and fetotoxicity of chromium in pregestationally exposed mice.

Four groups of 15 female Swiss albino mice were given Cr(VI), in the form of potassium dichromate, in drinking water at concentrations of 0, 250, 500, or 750 ppm. Treatment was maintained for 20 days, with the intent of covering the complete period required for development of an ovarian follicle. At the end of the 20 day treatment period, females were mated with untreated adult males; the day a vaginal plug was found was considered gestation day 0. Ten females from each group were randomly selected for sacrifice and evaluation on gestation day 19.

Water consumption in the control group averaged 8.52 $\pm$ 0.21 ml/mouse/day. For other groups, water consumption data were not provided, but consumed doses of Cr(VI) were determined as presented in Table C10 below.

Table C10. Consumed doses of Cr(VI) as potassium dichromate in drinking water in Junaid et al. (1996a) study

Cr(VI) concentration in water	0 ppm	250 ppm	500 ppm	750 ppm
Dose as mg Cr(VI)	0	1.9 $\pm$ 0.02	3.56 $\pm$ 0.03	5.23 $\pm$ 0.07

Gestational weight gain was not significantly affected in the 250 or 500 ppm groups. For these two groups, the numbers of implantations per litter and live fetuses per litter decreased with increasing Cr(VI) concentration, reaching statistical significance at 500 ppm ($p<0.05$ for both endpoints). Conversely, the rates of pre-implantation loss and resorptions per litter increased with increasing Cr(VI) concentration, reaching statistical significance at 500 ppm ($p<0.05$ for both endpoints). Post-implantation loss was significantly increased at both concentrations in a concentration-dependent manner ($p<0.05$ in all cases). Mean fetal weight and CRL were significantly decreased with increasing Cr(VI) concentration ($p<0.05$ for both endpoints at both concentrations). Placental weight was significantly decreased compared to controls at 250 ppm, but significantly increased at 500 ppm ($p<0.05$ in both cases).

Table C11. Developmental toxicity in Swiss mice - dams exposed via drinking water prior to mating. Junaid, et al. (1996b)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
Maternal wt gain (g)	14.40 $\pm$ 1.01	13.43 $\pm$ 0.50	12.38 $\pm$ 0.49	1.7 $\pm$ 0.93
Live fetuses/litter	7.7 $\pm$ 0.74	5.6 $\pm$ 0.50	3.4 + 0.24*	0
Fetal wt (g)	1.59 $\pm$ 0.04	1.11 $\pm$ 0.04*	0.97 + 0.03*	-
Placental wt (g)	0.137 $\pm$ 0.003	1.128 $\pm$ 0.005*	0.223 $\pm$ 0.005*	-
Resorptions/litter	0	1. 20 $\pm$ 0.44	2.0 + 0.31*	0
Post-implantation loss %	0	17.51 $\pm$ 2.22*	36.66 $\pm$ 4.94*	0

*$p < 0.05$

The 500 ppm group showed significant increases in the frequencies of "sub-dermal hemorrhagic patches," "kinky tail," "short tail;" and reduced parietal, inter-parietal, and caudal ossification ($p<0.05$ in all cases). A significant increase in the frequency of reduced caudal ossification was also observed in the 250 ppm group ($p<0.05$).

Chromium levels were determined in the maternal blood for all controls and animals exposed to all three Cr(VI) concentrations. All treated groups showed a significant increase over controls in the levels of Cr(VI) present in maternal blood ($P<0.05$ at all concentrations). The mean Cr(VI) level found at 750 ppm was significantly greater than in any other group ($p<0.05$ in all cases). As none of the 750 ppm animals were pregnant, there are no data on placental or fetal levels for this group. Hexavalent chromium levels in placentas increased in a concentration-dependent manner, with a significant increase over controls at 250 ppm ($p<0.05$), and significant increases over controls or the 250 ppm group at 500 ppm ($p<0.05$ at both concentrations). The increase in fetal Cr(VI) levels reached statistical significance only at 500 ppm ($p<0.05$ from controls or 250 ppm).

C.3. Integrative evaluation for developmental toxicity

C.3.1. Human data

A case-control study (Aschengrau et al., 1993) of drinking water quality and occurrence of late adverse pregnancy outcome did not find any significant associations between chromium levels and any pregnancy parameter, although the odds ratio for stillbirth was somewhat elevated. A descriptive geographical study (Eizaguirre-Garcia et al., 2000) of congenital anomalies in neighborhoods with areas of substantial ground contamination by chromium waste did not identify a teratogenic effect of the chromium waste. The study design had little potential to find an effect: the measure of chromium exposure was radial proximity to a chromium processing factory, and the measure of outcome was total number of congenital malformations observed over an eight year period, unadjusted for maternal age, a major determinant of malformation risk, or other pollutants.

C.3.2. Animal data

Available data on the potential developmental toxicity of Cr(VI) in experimental animals are from developmental studies with gestational exposure alone as well as studies with pre-conception exposures alone. Five developmental toxicity studies with gestation exposures were performed by the oral route (drinking water) – one in rats (Elsaieed and Nada, 2002), and four in mice (Trivedi et al., 1989; Junaid et al., 1995; Junaid et al., 1996a; De Flora et al., 2006). Most of these studies gave Cr(VI) in the form of potassium dichromate, although (Elsaieed and Nada, 2002) used potassium chromate. The De Flora et al. (2006) study, which also used sodium dichromate dihydrate, focused solely on the endpoint of micronuclei in hematopoietic cells; it also gave both potassium dichromate and sodium dichromate dihydrate by i.p. injection to additional groups of mice. Five studies had maternal exposure to Cr(VI) restricted to the pre-mating period, with no gestational exposure (Trivedi et al., 1989; Kanojia et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Kanojia et al., 1998).

Relevant data from the gestational exposure studies are summarized in Table C12.

Table C12. Animal studies of developmental toxicity: gestational exposure

Reference	Study design	Maternal toxicity	Developmental toxicity
Elsaieed & Nada (2002)	Rat 0, 50 ppm, GD 6–15 potassium chromate 10 ♀/group	↓ gestational wt gain[1]	↑ pre- and post implantation loss, resorption frequency, dead fetuses ↓ fetal weights ↑ visceral and skeletal anomalies, placental lesions

Table C12. (continued)

Junaid et al. (1996a)	Mouse 0, 250, 500, or 750 ppm, GD 6–18 potassium dichromate 10 ♀/group	No deaths or clinical signs ↓ wt gain at 500 & 750 ppm[2]	↑ resorption sites/litter, all treated groups ↓ fetuses/litter, fetal wts at 500 & 750 ppm ↑ post-implantation loss at 500 & 750 ppm ↑ ext anomalies at 750 ppm ↑ skeletal anomalies at 500 & 750 ppm
Junaid et al. (1995)	Mouse 0, 250, 500, or 750 ppm, GD 14–19 potassium dichromate 10 ♀/group	↓ wt gain at 500 & 750 ppm[3]	↑ post-implantation loss at 500 & 750 ppm ↑ placental wts at 500 & 750 ppm ↓ fetal wts & CRLs, all treated groups ↑ ext anomalies at 750 ppm ↑ skeletal anomalies, all treated groups
Trivedi et al. (1989)	Mouse 0, 250, 500, or 1000 ppm, GD 0–18 potassium dichromate 10 ♀/group	No deaths, or clinical or behavioral signs ↓ wt gain at 500 & 1000 ppm	No live litters or implantation sites at 1000 ppm ↓ fetuses/litter at 500 ppm ↑ pre-implantation loss at 500 ppm ↑ resorptions & post-implantation loss at 250 & 500 ppm ↓ fetal wts & CRLs at 250 & 500 ppm ↑ ext anomalies at 500 ppm ↑ skeletal anomalies at 250 & 500 ppm
De Flora et al. (2006)	Mouse micronuclei study 0, 5, 10 ppm, GD 0–18 sodium dichromate dihydrate, or potassium dichromate 5 ♀/group	No clastogenic effects in bone marrow No other systemic effects reported	No effects on litter size or fetal weights No clastogenic effects in blood or liver with oral exposure. (Clastogenic effects in blood or liver seen with i.p. exposure)

[1] 40% reduction in maternal weight gain associated with 85% reduction in mean litter weight
[2] 8% reduction in maternal weight gain associated with 30% reduction in mean litter weight at 500 ppm, 24% reduction in maternal weight gain associated with 34% reduction in mean litter weight at 750 ppm
[3] 11% reduction in maternal weight gain associated with 37% reduction in mean litter weight at 500 ppm, 26% reduction in maternal weight gain associated with 47% reduction in mean litter weight at 750 ppm

All of the gestational studies reported statistically significant effects of oral Cr(VI) exposure on fetal viability, weight, and external and skeletal anomalies. Thus, the effects occurred in three studies in mice (Trivedi et al., 1989; Junaid et al., 1995; Junaid et al., 1996a) and the one study in rats (Elsaieed and Nada, 2002). Effective concentrations in drinking water ranged from 50 to 750 ppm; no live fetuses or implantation sites were seen in dams exposed to 1000 ppm Cr(VI) (Trivedi et al., 1989). For these studies, no maternal deaths or clinical or behavioral signs were reported. Decreased maternal gestational weight gain at doses that were associated with developmental effects was reported. However, since maternal weight gain during gestation is primarily the result of the increasing feto-placental weights during gestation, it is noteworthy that the decreases in maternal weight gain were proportionately less than the decreases in mean litter weights, suggesting that the maternal weight gain decrement was secondary to reduced litter weights resulting from Cr(VI) exposure.

The De Flora et al. (2006) study was not a standard developmental toxicity study, but rather was designed to evaluate evidence for genotoxicity (micronuclei). This was not found in fetal liver or blood with oral exposure up to 10 ppm Cr(VI) in water. However, a single i.p. injection at 50 mg/kg body weight on GD 17 resulted in significant increases in the frequencies of micronucleated polychromatic erythrocytes in fetal liver and blood, as well as maternal bone marrow. None of these treatments affected litter size or fetal weights.

It is a generally accepted principle of developmental toxicology that relevant adverse developmental effects may result from exposure of either parent prior to conception (e.g., (U.S. EPA, 1991). Thus, studies of the impact of pre-conceptual exposure to Cr(VI) on offspring are also presented in this report. These are given in Table C13.

Table C13. Animal studies of developmental toxicity: pre-mating maternal exposure

Reference	Study design	Maternal toxicity	Developmental toxicity
Kanojia et al. (1998)	Rat 0, 250, 500, 750 ppm, 3 months prior to mating; treatment ended before mating potassium dichromate 10 ♀/group	↑ mortality at 500 & 750 ppm ↓ body wt & gestational wt gain at 500 & 750 ppm[1]	↓ corpora lutea, implantations, live fetuses/litter at 500 & 750 ppm ↑ resorption frequency at 500 & 750 ppm ↑ pre- & post-implantation loss, all treated groups ↓ fetal wt, all treated groups ↓ placental wt & CRLs at 500 & 750 ppm ↑ external and skeletal anomalies, all treated groups
Kanojia et al. (1996)	Rat 0, 250, 500, 750 ppm; 20 days prior to mating; treatment ended before mating potassium dichromate 20 ♀/group (10 bred, 10 for cycle studies)	↓ wt gain, all concentrations[2]	↓ corpora lutea, implantations, at 500 & 750 ppm ↑ pre-implantation loss at 500 & 750 ppm ↑ resorption frequency, post-implantation loss, & placental wts, all treated groups ↓ live fetuses/litter, all treated groups ↑ external anomalies, 750 ppm ↑ skeletal anomalies, 500 & 750 ppm No effect on fetal wts or CRL

Table C13. (continued)

Al-Hamood, et al. (1998)	Mouse 0, 1000 ppm; gd 12 - lactation potassium dichromate 25 pregnant ♀s/group litters culled to 8 on PND 0 PND 60 ♀ offspring mated with untreated ♂s	No data reported	<u>In F1 offspring</u>: delayed vaginal opening ↓ pregnancy rate, implantations & viable fetuses No effects on body wt, ovarian wt, or uterine wt
Elbetieha and Al-Hamood (1997)	Mouse 0, 2000, 5000 ppm; 12 weeks prior to mating; treatment ended before mating potassium dichromate 11-18 ♀/group	No data reported	↓ implantations, viable fetuses at 2000 & 5000 ppm ↑ resorptions at 2000 & 5000 ppm
Junaid et al. (1996b)	Mouse 0, 250, 500, 750 ppm; 20 days prior to mating; treatment ended before mating potassium dichromate 10 ♀/group	No clinical or behavioral signs of toxicity No gestational wt gain at 750 ppm	No live fetuses, implantation sites or resorptions, 750 ppm ↓ corpora lutea at 750 ppm ↓ implantations and live fetuses/litter at 500 ppm ↑ pre-implantation loss and resorptions/litter at 500 ppm ↑ post-implantation loss, 250 & 500 ppm ↓ fetal wt & CRLs, 250 & 500 ppm ↓ placental wt, 250 ppm ↑ placental wt, 500 ppm ↑ ext anomalies at 500 ppm ↑ skeletal anomalies at 250 & 500 ppm

[1] 17% reduction in gestational weight gain associated with 47% reduction in mean litter weight at 500 ppm, 22% reduction in gestational weight gain associated with 73% reduction in mean litter weight at 750 ppm

[2] 7% reduction in gestational weight gain associated with 18% reduction in mean litter weight at 250 ppm, 13% reduction in gestational weight gain associated with 61% reduction in mean litter weight at 500 ppm, 21% reduction in gestational weight gain associated with 91% reduction in mean litter weight at 750 ppm

Adverse effects were seen in the offspring of dams that were exposed to Cr(VI) before mating to unexposed males in several female reproductive toxicity studies (Trivedi et al., 1989; Kanojia et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Kanojia et al., 1998). Observed fetal effects were similar to those found with gestational exposures, including: decreased viability of the conceptus (both pre- and post-implantation), decreased fetal weights and CRL, changes in placental weights (decreased or increased), and increased frequencies of external and skeletal anomalies. These effects were seen at Cr(VI) concentrations in drinking water ranging from 250–750 ppm, with numbers and severity of effects increasing with increasing concentration.

In some studies (Trivedi et al., 1989; Kanojia et al., 1996; Junaid et al., 1996b; Kanojia et al., 1998), maternal and fetal levels of Cr(VI) were determined and found to be elevated in previously-exposed dams and their offspring. Hence, it would appear that Cr(VI)

accumulated in maternal tissues during treatment, remained during the (untreated) mating period, and then crossed the placenta into fetuses during gestation.

D. FEMALE REPRODUCTIVE TOXICITY

D.1. Human female reproductive toxicity studies

There are few epidemiological data on the female reproductive toxicity of Cr(VI). There are reports of spontaneous abortion in spouses of welders exposed to Cr(VI) (Bonde, 1993; Hjollund et al., 1995; Hjollund et al., 2000). Because the hypothesis for the spontaneous abortion is that they are male mediated (as found in animals), and there is not evidence of excess female exposure to Cr(VI) through for example handling of husband's clothing, these studies are discussed with other studies of male reproductive toxicity in Section E.1.

For direct exposure to Cr(VI), there are two occupational studies involving manufacturing of chromium compounds reported in Russian language journals (Shmitova, 1978; Shmitova, 1980). These papers have been described by ATSDR (2000). The Office of Environmental Health Hazard Assessment obtained abstracts of the studies, but reports the ATSDR descriptions because they apparently had available to them the translation of the Russian papers.

> "The effect of chromium(VI) on the course of pregnancy and childbirth was studied in women employees at a dichromate manufacturing facility in Russia. Complications during pregnancy and childbirth (not further described) were reported in 20 of 26 exposed women who had high levels of chromium in blood and urine, compared with 6 of 20 women in the control group. Toxicosis (not further described) was reported in 12 exposed women and 4 controls. Postnatal hemorrhage occurred in four exposed and two control women (Shmitova, 1980). Similar results were reported in a more extensive study of 407 women who worked at a factory producing chromium compounds (not otherwise specified) compared with 323 controls. The frequency of birth complications was 71.4% in a subgroup of highly exposed women, 77.4% in a subgroup of women with a lower level of exposure, and 44.2% in controls. Toxicosis in the first half of pregnancy occurred in 35.1% of the high exposure group, 33.3% of the low exposure group, and 13.6% of the controls. The frequency of post-natal hemorrhage was 19.0% for the high exposure group and 5.2% in controls (Shmitova, 1978). Because these studies were generally of poor quality and results were poorly reported, no conclusions can be made regarding the potential for chromium to produce reproductive effects in humans." (ATSDR, 2000)

D.2. Animal female reproductive toxicity studies

Available data on the potential female reproductive toxicity of Cr(VI) in experimental animals are provided in seven reproductive studies performed by the oral route (drinking water), two in rats (Kanojia et al., 1996; Kanojia et al., 1998), and five in mice (Trivedi et al., 1989; Murthy et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Al-Hamood et al., 1998). A study in rats in which exposure of pups occurred only via lactation from dams treated with Cr (VI) via drinking water is also included because effects on the developing female reproductive system in pups were investigated (Banu et al., 2008).

Rat, oral (drinking water)

Kanojia, et al. (1998). Embryo and fetotoxicity of hexavalent chromium: a long-term study.

Female rats of the "Druckery" strain were given potassium dichromate in drinking water to Cr(VI) concentrations of 0, 250, 500, or 750 ppm. Treatment was begun when animals were 50 days of age, and continued for a period of three months. At the end of the treatment period, each female was caged for mating with a single, untreated male. The presence of sperm in a daily vaginal smear was considered to represent day 0 of pregnancy. Ten dams from each group were sacrificed for Caesarian section on gestation day 19.

During the first two weeks of treatment, mortality was 15% among 500 ppm animals and 10% in the 750 ppm Cr(VI) group. Based on water intake data, Cr(VI) intake was determined as presented in Table D1 below.

Table D1. Consumed doses of Cr(VI) as potassium dichromate by rats in drinking water in Kanojia et al. (1998)

Cr(VI) concentration in water	**0 ppm**	**250 ppm**	**500 ppm**	**750 ppm**
Dose as mg Cr(VI)/rat/day	0	5.57	10.18	13.56

At the end of the 90-day treatment period, all treated female rats were found to be acyclic, and in a persistent diestrous phase. During the subsequent 15–20 day mating period, estrous cycles returned, and animals began to mate. Significant ($p<0.05$) lengthening of estrous cycles was seen in all treated groups, relative to controls, and occurred in a concentration-dependent manner.

Mating and fertility indices decreased with increasing Cr(VI) concentration as shown in Table D2 below. No statistical analysis was presented for these data.

Table D2. Mating and fertility indices for female rats exposed to Cr(VI) for 90 days prior to mating - drinking water study of Kanojia et al. (1998)

Cr(VI) concentration in water	0 ppm	250 ppm	500 ppm	750 ppm
No. of animals per group	10	10	10	10
Mating index (%)	100	70	60	40
Fertility index (%)	98	67	58	50
Estrous cycle length	5.15±0.21	6.36±0.44*	7.18±0.46*	8.66±0.63*

Maternal body weight at the end of gestation and maternal weight gain during gestation decreased with increasing concentration of Cr(VI); the changes reached statistical significance ($p<0.05$) for both endpoints at the two highest concentrations. The numbers of corpora lutea, implantations, and live fetuses per litter all decreased with increasing Cr(VI) concentration, with differences among groups reaching statistical significance ($p<0.05$) at 500 and 750 ppm. Resorption frequency increased with increasing concentration of Cr(VI), reaching statistical significance ($p<0.05$) at 500 and 750 ppm. The frequencies of both pre- and post-implantation loss were significantly increased relative to controls in all treated groups ($p<0.05$), in a concentration-dependent manner.

Table D3. Female reproductive toxicity data from the drinking water study of Kanojia et al. (1998)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
Corpora lutea	10.50 ± 0.56	9.56 ± 0.73	8.33 ± 0.49*	7.13 ± 0.60*
No. implantations	9.83 ± 0.92	8.32 ± 0.82	6.86 ± 0.42*	5.70 ± 0.72*
Live fetuses/litter	9.30 ± 0.92	7.30 ± 0.53	5.45 ± 0.42*	4.16 ± 0.31*
Resorptions/litter	0.53 ± 0.25	1.02 ± 0.30	1.41 ± 0.37*	1.67 ± 0.34*
Pre-implantation loss %	6.83 ± 0.63	12.97 ± 0.75*	17.64 ± 0.45*	20.05 ± 0.45*
Post-implantation loss %	5.39 ± 0.35	12.25 ± 0.63*	20.25 ± 0.54*	22.80 ± 0.91*

*$p < 0.05$

Chromium concentrations in maternal blood, placenta, and fetal tissues increased significantly with increasing Cr(VI) intake ($p<0.05$ among all groups). When chromium content was considered as a ratio of placenta to maternal blood or fetus to placenta, all treated groups were significantly increased relative to controls ($p<0.05$), but there was no significant additional increase with increasing intake.

Kanojia et al. (1996). Chromium induced teratogenicity in the female rat.

Adult Swiss albino female rats were given potassium dichromate in drinking water at concentrations selected to provide 0, 250, 500 or 750 ppm Cr(VI). Based on water intake data collected during the study, these concentrations were determined to have provided

doses of 0, 6.44, 12.2, and 15.28 mg/kg-day. The animals were treated for 20 days. After the cessation of treatment, females were caged (1:1) with untreated males for mating. Ten pregnant females from each group were sacrificed on GD 19 (day 0 = day sperm detected in vaginal smear) for examination of reproductive organs and uterine contents. An additional 10 rats per group were not bred, but had daily vaginal smears taken for 12 consecutive estrous cycles, in order to determine cycle length.

For the non-bred animals, estrous cycles increased in length for all groups, reaching a statistically significant ($p<0.05$) difference from controls for the 750 ppm group.

For bred animals, both mating and fertility indices were strongly reduced with increasing Cr(VI) concentration (see Table D4, below).

Table D4. Mating and fertility indices in Swiss albino rats receiving Cr(IV) in drinking water - data from Kanojia et al. (1996)

Cr(VI) concentration in water	**control**	**250 ppm**	**500 ppm**	**750 ppm**
Number of animals per group	10	10	10	10
Mating Index (%)	100	80	70	40
Fertility Index (%)	96	75	57	31
Estrous cycle length (days)	5.2±0.2	5.4±0.7	5.7±0.6	7.1±0,5*

*$p<0.05$

Relative to controls, maternal weight gain was significantly decreased at all concentrations of Cr(VI), in a concentration-related manner. The numbers of corpora lutea decreased with increasing Cr(VI) concentration, showing statistically significant reductions ($p<0.05$) from controls in the 500 and 750 ppm groups. Implantation frequency was significantly ($p<0.05$) lower than controls for the 500 and 750 ppm groups. The number of live fetuses per litter showed a concentration-dependent decrease (from a mean of 9.11 in controls to 1.21 at 750 ppm). Differences from controls were statistically significant ($p<0.05$) at all Cr(VI) concentrations. Conversely, resorption frequency increased with increasing Cr(VI) concentration; a change that was significantly different from controls ($p<0.05$) at all concentrations tested. Pre-implantation loss was significantly greater ($p<0.05$) in treated than control groups for the 500 and 750 ppm groups. Post-implantation loss showed a striking and significant ($p<0.05$) increase with increasing Cr(VI) concentration, at all concentrations.

Table D5. Female reproductive data from Kanojia et al. (1996)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
Corpora lutea	10.02 ± 0.91	9.81 ± 0.95	7.13 ± 0.61*	4.43 ± 0.50*
No. implantations	9.51 ± 0.96	9.61 ± 0.83	5.91 ± 0.39*	2.27 ± 0.36*
Live fetuses/litter	9.11 ± 0.87	8.29 ± 0.0.93*	4.12 ± 0.51*	1.21 ± 0.13*
Resorptions/litter	0.40 ± 0.24	1.09 ± 0.34*	1.72 ± 0.23*	1.03 ± 0.29*
Pre-implantation loss %	5.08 ± 0.65	2.03 ± 0.31	17.11 ± 2.13*	48.75 ± 5.81*
Post-implantation loss %	4.20 ± 0.41	13.73 ± 1.57*	30.28 ± 4.19*	46.69 ± 5.21*

*$p < 0.05$

Chromium levels were measured in maternal blood, placenta, and in fetuses. Fetuses of the 500 and 750 ppm groups showed significant increases in chromium content ($p<0.05$), as compared to controls. Maternal blood and the placentas from all treated groups were significantly higher in chromium content ($p<0.05$) as compared to controls. While the transfer ratio from placenta to fetus did not differ among treated groups, the transfer ratio from the maternal circulation to the placenta was greater for the 750 ppm group than for the other two treated groups.

Banu et al., (2008). Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats.

Pregnant Wistar rats were divided into three groups of 18. All dams were allowed to deliver their litters normally, but litters were culled to four female pups per dam on the day of birth. During lactation days 1-20, control dams were given untreated drinking water, while treated groups of dams were given either 200 mg potassium dichromate/L drinking water or the same concentration of potassium dichromate with the addition of 500 mg ascorbate/L. All four female offspring from each of six dams were sacrificed for evaluation at one of three time points: postnatal day (PND) 21 (weaning), PND 45, or PND 65. Blood and ovaries were collected.

Chromium levels in plasma and ovaries of exposed offspring were significantly higher ($p < 0.05$) than in control offspring at all time points, though levels declined at the later time points. Vitamin C ameliorated Cr levels to an increasing extent at the later time points, returning them to control values by PND 65 in both plasma and ovarian tissue.

The time of vaginal opening, which serves as a marker for the onset of puberty, was 55 ± 2.5 days in Cr(VI) exposed female offspring, as compared to 33 ± 1.5 days in controls. The difference was statistically significant at $p < 0.05$. Offspring co-exposed to vitamin C and Cr(VI) showed vaginal opening at 35 ± 1.4 days, which was not significantly different from controls.

Significant lengthening of the estrous cycle, specifically diestrous, was significantly lengthened in Cr(VI)-exposed female offspring ($p < 0.05$) as compared to controls. The lengths of other phases of the estrous cycle were not affected.

Numbers of ovarian follicles were reduced in Cr(VI)-exposed offspring compared to controls at PND 21, 45, and 65 ($p < 0.05$). Not all follicle types were affected at all time points (see table D6 below). Co-exposure to Vitamin C mitigated the effects of Cr(VI).

Table D6. Effects of lactational Cr(VI) exposure on populations of ovarian follicles

Age and experimental group	Primordial follicles	Primary follicles	Secondary follicles	Antral follicles
PND21 - control	97.8 ± 7.2	52.8 ± 6.4	22.8 ± 1.6	15.8 ± 3.1
PND 21 - Cr(VI)	76.3 ± 4.6*	35.5 ± 4.4*	14.6 ± 0.6*	0
PND 45 - control	133.5 ± 13.0	34.8 ± 5.1	11.2 ± 1.8	7.8± 1.2
PND 45 - Cr(VI)	91.8 ± 8.2*	22.7 ± 4.6*	5.8 ± 0.8*	1.2 ± 0.5*
PND 65 - control	112.6 ± 9.9	32.3 ± 3.7	9.5 ± 1.7	8.0 ± 1.5
PND 65 - Cr(VI)	90.8 ± 7.2*	21.78 ± 3.4*	8.8 ± 1.5	7.4 ± 2.1

* $p < 0.05$

Circulating levels of steroid and pituitary hormones were also evaluated at all three time points. Estradiol (E2), testosterone (T), progesterone (P4), follicle stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL) levels all showed significant changes from control levels ($p < 0.05$) for at least some time points in Cr(VI)-exposed offspring (see tables D7 and D8, below). Of all the hormones measured, only luteinizing hormone (LH) showed no effect of treatment. Co-exposure to Vitamin C mitigated the effects of Cr(VI).

Table D7. Effects of lactational Cr(VI) exposure on circulating levels of steroid hormones

Age and experimental group	E2 (pg/ml)	T (ng/ml)	P4 (ng/ml)
PND 21 - control	30.2 ± 3.5	0.87 ± 0.07	4.21 ± 0.45
PND 21 - Cr(VI)	11.1 ± 1.2*	0.35 ± 0.06*	1.90 ± 0.18*
PND 45 - control	43.8 ± 4.0	0.94 ± 0.07	6.42 ± 0.45
PND 45 - Cr(VI)	24.1 ± 2.3	0.45 ± 0.04*	3.70 ± 0.68*
PND 65 - control	40.9 ± 3.5	1.07 ± 0.08	9.01 ± 0.85
PND 65 - Cr(VI)	27.3 ± 2.1*	0.56 ± 0.05*	5.21 ± 0.68*

* $p < 0.05$

Table D8. Effects of lactational Cr(VI) exposure on circulating levels of pituitary hormones

Age and experimental group	ESH (ng/ml)	GH (ng/ml)	PRL (ng/ml)
PND 21 - control	12.4 ± 1.32	204.2 ± 19.0	25.5 ± 2.1
PND 21 - Cr(VI)	19.8 ± 0.98*	98.7 ± 10.3*	16.2 ± 1.1*
PND 45 - control	10.0 ± 31.0	274.2 ± 31.0	38.4 ± 3.17
PND 45 - Cr(VI)	16.7 ± 1.78*	145.3 ± 15.9*	22.3 ± 2.50*
PND 65 - control	9.30 ± 0.75	150.0 ± 16.8	35.2 ± 3.5
PND 65 - Cr(VI)	10.1 ± 1.28	106.4 ± 13.0*	18.4 ± 1.08*

* $p < 0.05$

Additional *in vitro* experiments were carried out using a spontaneously immortalized rat granulosa cell line (SIGC). Incubating SIGC cells for 12 or 24 hours with 12.5 μg (IC_{50}) potassium dichromate decreased mRNA expression of steroidogenic acute regulatory protein, steroidogenic factor-1, 17β-hydroxysteroid dehydrogenases-1 and -2, FSH-receptor, LH-receptor, estrogen receptors-α and -β. These effects were alleviated by pre-incubation with vitamin C.

Mouse, oral (drinking water)

Al-Hamood, et al. (1998). Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds.

Groups of 25 timed-pregnant BALBC mice (plug day = day 0) were give 0 or 1000 ppm potassium dichromate in drinking water from gestation day 12 through lactation day 20. On the day of birth, litters were adjusted to eight pups each. From PND 20 on, female pups were examined daily for vaginal opening as an indicator of puberty. At PND 60, females were caged in groups of three with a single untreated male of proven fertility. Females were left in mating cages for 10 days, to cover two complete estrous cycles. One week after the end of the mating period, the females were sacrificed for examination of their uterine contents.

While body weights of females exposed pre- and postnatally to Cr(VI) were not significantly different from controls, the time of vaginal opening was delayed by approximately three days ($p<0.001$). The pregnancy rate was reduced to 63.5% in treated females, as compared to 100% for controls (significant at $p<0.025$). The numbers of implantations and viable fetuses were both significantly reduced for treated females ($p<0.05$ for both endpoints). Three resorptions were observed among treated females, while none occurred in the control group.

Table D9. Female reproductive toxicity indicators in BALBC mice exposed to Cr(VI) in drinking water by from Al-Hamood et al. (1998)

Parameter/treatment	control	1000 ppm Cr(VI)
No. females	12	22
No. pregnant (%)	12 (100)	14 (63.6)
No. implantations	10.25 $\pm$ 1.48	9.07 $\pm$ 1.14*
No. viable fetuses	10.25 $\pm$ 1.48	8.85 $\pm$ 1.56*
No. resorptions	0	3

* $p < 0.05$

Female reproductive toxicity indicators in BALBC mice exposed to Cr(VI) in drinking water by

Additional animals were exposed pre- and postnatally as described above, and sacrificed on PND 50 for measurements of body weight and reproductive organ weights. In these groups there were 12 controls and nine females that had been exposed pre- and postnatally to 1000 ppm potassium dichromate. No effect of treatment was found on body weight, ovarian weight, or uterine weight.

The delay in onset of puberty, as evidenced by delayed time of vaginal opening, was taken by the authors to indicate an effect of Cr(VI) on the hypothalamic-pituitary-gonadal axis and delayed estrogen secretion.

Elbetieha, et al. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility.

Adult female Swiss mice were given 0, 2000, or 5000 mg/L (ppm) potassium dichromate for 12 weeks. At the end of treatment, females were housed with untreated, proven fertile males, at a ratio of 3:1, for a period of ten days (presumed to encompass at least two estrous cycles). The animals were sacrificed at one week following the end of mating for examination of their uterine contents. Additional control (n=8) and treated (n=10, 5000 ppm only) animals were not mated, but were sacrificed at the end of the exposure period for determination of body and organ weights.

The numbers of pregnant animals as a fraction of total mated females for the control, 2000, and 5000 ppm groups were as follows: 17/18, 14/15, and 9/11. Relative to controls, the numbers of implantations per litter were significantly reduced in both the 2000 ppm ($p<0.01$) and 5000 ppm ($p<0.05$) groups. The number of viable fetuses was also significantly reduced at both 2000 ppm ($p<0.05$) and 5000 ppm ($p<0.01$). The number of litters with resorptions was significantly increased at 2000 ppm ($p<0.01$) and 5000 ppm ($p<0.005$).

Table D10. Female reproductive data from Elbetieha and Al-Hamood (1997)

Parameter/Cr(VI) concentration	Control	2000 mg/L	5000 mg/L
No. pregnant/No. ♀s	17/18	14/15	9/11
No. implantations	9.00 ± 1.36	7.35 ± 1.54**	7.44 ± 1.50*
Live fetuses/litter	8.76 ± 1.39	6.55 ± 2.18*	5.88 ± 2.47**
Litters with resorptions	2/18 (11%)	8/15 (53%)**	7/11 (63%)
Total resorptions	4	37	14

*p < 0.05; **p < 0.01

Body weights and uterine weights of treated animals were not affected by treatment, but ovarian weights were significantly increased ($p<0.05$) with exposure to Cr(VI) compound at 5000 ppm.

The authors suggest that their data are consistent with a chromium-induced disturbance of reproductive endocrine functions, and that the increased resorptions were likely due to modification of the uterine lining prior to arrival of the embryo.

Junaid et al. (1996b). Embryo- and fetotoxicity of chromium in pregestationally exposed mice.

Four groups of 15 female Swiss albino mice were given Cr(VI), in the form of potassium dichromate, in drinking water at concentrations of 0, 250, 500, or 750 ppm. Treatment was maintained for 20 days, with the intent of covering the complete period required for development of an ovarian follicle. At the end of the 20 day treatment period, females were mated with untreated adult males; the day a vaginal plug was found was considered gestation day 0. Ten females from each group were randomly selected for sacrifice and evaluation on gestation day 19.

Water consumption in the control group averaged 8.52 ± 0.21 ml/mouse/day. For other groups, water consumption data were not provided, but consumed doses of Cr(VI) were determined as presented in Table D11 below.

Table D11. Consumed doses of Cr(VI) as potassium dichromate in drinking water in Swiss mice study of Junaid et al. (1996b)

Cr(VI) concentration in water	0 ppm	250 ppm	500 ppm	750 ppm
Dose as mg Cr(VI)/mouse/day	0	1.9 ± 0.02	3.56 ± 0.03	5.23 ± 0.07

No clinical or behavioral signs of toxicity were noted during the treatment phase of the study. The 750 ppm group had significantly fewer corpora lutea than any of the other groups ($p<0.05$ in all cases). This group had no implantation sites, no resorptions, and no live fetuses. In the absence of any pregnancies, this group showed no gestational weight gain.

Gestational weight gain was not significantly affected in the 250 or 500 ppm groups. For these two groups, the numbers of implantations per litter and live fetuses per litter decreased with increasing Cr(VI) concentration, reaching statistical significance at 500 ppm ($p<0.05$ for both endpoints). Conversely, the rates of pre-implantation loss and resorptions per litter increased with increasing Cr(VI) concentration, reaching statistical significance at 500 ppm ($p<0.05$ for both endpoints). Post-implantation loss was significantly increased at both concentrations in a concentration-dependent manner ($p<0.05$ in all cases).

Table D12. Female reproductive toxicity data from Junaid, et al. (1996b)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
Corpora lutea	7.9 ± 1.01	7.4 ± 0.50	7.3 ± 0.37	4.4 ± 0.50*
No. implantations	7.7 ± 0.74	6.8 ± 0.41	5.4 ± 0.27*	0
Live fetuses/litter	7.7 ± 0.74	5.6 ± 0.50	3.4 ± 0.24*	0
Resorptions/litter	0	1. 20 ± 0.44	2.0 ± 0.31*	0
Pre-implantation loss %	2.77 ± 1.21	8.38 ± 3.53	24.79 ± 2.17*	100
Post-implantation loss %	0	17.51 ± 2.22*	36.66 ± 4.94*	0

*$p < 0.05$

At the time of sacrifice, chromium levels were determined in the blood of five dams from each group, and one fetus plus its placenta per litter. All treated groups showed a significant increase over controls in the levels of Cr(VI) present in maternal blood ($P<0.05$ at all concentrations). The mean Cr(VI) level found at 750 ppm was significantly greater than in any other group ($p<0.05$ in all cases). As none of the 750 ppm animals were pregnant, there are no data on placental or fetal levels for this group. Cr(VI) levels in placentas increased in a concentration-dependent manner, with a significant increase over controls at 250 ppm ($p<0.05$), and significant increases over controls or the 250 ppm group at 500 ppm ($p<0.05$ at both concentrations). The increase in fetal Cr(VI) levels reached statistical significance only at 500 ppm ($p<0.05$ from controls or 250 ppm).

Murthy et al. (1996). Ovarian dysfunction in mice following chromium (IV) exposure.

Two sets of adult Swiss albino female mice were exposed to potassium dichromate in drinking water in a study of ovarian function. Set I consisted of four groups of 30 mice each that were given potassium dichromate at concentrations selected to provide 0, 250, 500, or 750 ppm Cr(VI) for 20 days, in order to follow a complete cycle of folliculogenesis. Set II animals, which were divided into four groups of 10 mice each, were given potassium dichromate at concentrations to provide 0, 0.05, 0.5, or 5 ppm Cr(VI) for 90 days prior to electron microscopic studies of their ovaries.

In Set I mice, the numbers of small, medium, and large follicles were all reduced with potassium dichromate in a dose-related fashion. Statistically significant ($p<0.05$) reductions from controls were found for medium and large follicles in the 250 ppm group, as well as for small, medium, and large follicles in the 500 and 750 ppm groups. Estrous cycles were lengthened in a dose-related manner, which reached statistical significance at the high concentration of 750 ppm ($p<0.05$). Ten animals from each of the Set I dose groups were subjected to super-ovulation, and had their ova harvested for counting. The number of ova per mouse decreased with increasing dose, reaching statistical significance at 500 and 750 ppm ($p<0.05$).

Table D13. Female reproductive toxicity data from Murthy et al. (1996)

Parameter/Cr(VI) concentration	Control	250 ppm	500 ppm	750 ppm
No. small follicles	39.4 ± 0.5	36.2 ± 0.4	34.0 ± 0.3*	25.2 ± 0.4*
No. med follicles	9.8 ± 0.4	7.6 ± 0.2*	6.2 ± 0.3*	4.6 ± 0.2*
No. large follicles	7.6 ± 0.2	6.6 ± 0.2*	5.2 ± 0.2*	2.4 ± 0.24*
Estrous cycle length (days)	4.4 ± 0.6	4.7 ± 0.6	5.8 ± 0.4	7.7 ± 0.8*
No. ova/mouse	26.2 ± 1.1	25.4 ± 0.5	18.4 ± 1.2*	2.4 ± 0.9*

*$p < 0.05$

Among Set I animals, histology revealed no change from controls for the ovaries of the 250 ppm group. The 500 ppm group showed changes in follicular cells of some of the mature follicles. The most severe effects were found in the follicles of the high dose group (750 ppm). Ovaries of these animals showed a large number of atretic follicles; in a few cases, the ovaries were completely hemolytic.

Ultrastructure analysis of Set II animals revealed evidence for changes in the ovaries of the 5 ppm animals (the highest dose used in this set) such as damage to follicular cell membranes.

Trivedi et al. (1989). Embryotoxicity and fetotoxicity of orally administered hexavalent chromium in mice.

As previously described in Section C.2.1., groups of ten timed-pregnant female ITRC-bred albino mice were given Cr(VI) in drinking water at concentrations of 0, 250, 500, or 1000 ppm. Treatment began on gestation day 0 (the day a seminal plug was detected) until gestation day 18. Sacrifice for examination of uterine contents was performed on gestation day 19.

There was no maternal mortality observed during the study period, and no clinical or behavioral signs of toxicity were observed for dams in group. At the highest concentration of potassium dichromate (1000 ppm), no live litters or implantation sites were found. These animals had significantly lower body weight gain than controls over

the study period ($p<0.001$). Weight gain was also significantly decreased in the 500 ppm group ($p<0.01$).

Statistically significant increases in chromium levels were found in maternal blood of the 1000 ppm group ($p<0.001$), placentas at both 250 and 500 ppm ($p<0.001$), and fetuses of the 500 ppm group ($p<0.01$).

D.3. Integrative evaluation for female reproductive toxicity

D.3.1. Human data

The only studies showing unambiguous female exposures to Cr(VI) followed by observations of pregnancy outcome are two occupational studies of employees at chromate manufacturing facilities in Russia, published in Russian and found by ATSDR (2000) to be poorly reported. The Office of Environmental Health Hazard Assessment did not have access to translations of the full papers. Of women with high levels of chromium in blood and urine, relatively large proportions were reported to have complications during pregnancy as well as toxicosis compared to controls in both studies. These effects however were not further described, and other aspects of the studies were poorly reported. The ATSDR therefore noted that no conclusions could be made based on these studies.

D.3.2. Animal data

Available data on the potential female reproductive toxicity of Cr(VI) in experimental animals comes from reproductive studies performed by the oral route (drinking water), two in rats (Kanojia et al., 1996; Kanojia et al., 1998), and in mice (Trivedi et al., 1989; Murthy et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Al-Hamood et al., 1998). Findings of female reproductive toxicity from these studies are summarized in Table D14.

All of the studies in both species gave evidence for adverse effects of Cr(VI) on the female reproductive system at concentrations in drinking water ranging from 5 ppm (ovarian alterations at ultra-structural level) to 1000 ppm (complete pregnancy failure in all exposed dams). Exposure to more moderate concentrations (500–750 ppm), was associated with effects such as: decreased mating and fertility indices; decreased numbers of corpora lutea, implantation sites, and live fetuses/litter; and increased frequencies of pre- and post-implantation loss, as well as resorption sites. Treatment with Cr(VI) was associated with lengthening of the estrous cycle in both rats and mice (Kanojia et al., 1996; Murthy et al., 1996; Kanojia et al., 1998).

Table D14. Animal studies of female reproductive toxicity of Cr(VI): oral, drinking water

Reference	Study design	Systemic toxicity	Female reproductive toxicity
Kanojia et al. (1998)	Rat 0, 250, 500, 750 ppm, 3 months prior to mating; treatment ended before mating potassium dichromate 10 ♀/group	↑ mortality at 500 & 750 ppm ↓ body wt & gestational wt gain at 500 & 750 ppm	↑ length of estrous cycles, all treated groups ↓ mating & fertility indices, all treated groups (no statistical analysis) ↓ corpora lutea, implantations, live fetuses/litter at 500 & 750 ppm ↑ resorption frequency at 500 & 750 ppm ↑ pre- & post-implantation loss, all treated groups
Kanojia et al. (1996)	Rat 0, 250, 500, 750 ppm; 20 days prior to mating; treatment ended before mating potassium dichromate 20 ♀/group (10 bred, 10 for cycle studies)	↓ wt gain, all concentrations	↑ length of estrous cycles at 750 ppm ↓ mating & fertility indices, all treated groups (no statistical analysis) ↓ corpora lutea, implantations, at 500 & 750 ppm ↑ pre-implantation loss at 500 & 750 ppm ↑ resorption frequency, post-implantation loss, & placental wts, all treated groups ↓ live fetuses/litter, all treated groups
Al-Hamood, et al. (1998)	Mouse 0, 1000 ppm; GD 12 - lactation potassium dichromate 25 pregnant ♀s/group litters culled to 8 on PND 0 PND 60 ♀ offspring mated with untreated ♂s	No data reported	<u>In F1 offspring</u>: delayed vaginal opening ↓ pregnancy rate, implantations & viable fetuses No effects on body wt, ovarian wt, or uterine wt
Elbetieha and Al-Hamood (1997)	Mouse 0, 2000, 5000 ppm; 12 weeks prior to mating; treatment ended before mating potassium dichromate 11-18 ♀/group	No data reported	↓ implantations, viable fetuses at 2000 & 5000 ppm ↑ resorptions at 2000 & 5000 ppm ↑ ovarian wt at 5000 ppm No effect on body or ovarian wts

Table D14. (continued)

Junaid et al. (1996b)	Mouse 0, 250, 500, 750 ppm; 20 days prior to mating; treatment ended before mating potassium dichromate 10 ♀/group	No clinical or behavioral signs of toxicity No gestational wt gain at 750 ppm	No live fetuses, implantation sites or resorptions, 750 ppm ↓ corpora lutea at 750 ppm ↓ implantations and live fetuses/litter at 500 ppm ↑ pre-implantation loss and resorptions/litter at 500 ppm ↑ post-implantation loss, 250 & 500 ppm ↓ placental wt, 250 ppm ↑ placental wt, 500 ppm
Murthy et al. (1996)	Mouse A. 0, 250, 500, or 750 ppm; 20 days, or B. 0, 0.05, 0.5, 5 ppm; 90 days potassium dichromate A. 30 ♀/group B. 10 ♀/group	No data reported	A. ↑ length of estrous cycles, 750 ppm ↓ numbers of ova, 500 & 750 ppm ↓ small, medium, & large ovarian follicles ↑ atretic follicles, hemolytic ovaries, 750 ppm B. Ultrastructure analysis revealed ovarian changes at 5 ppm
Trivedi et al. (1989)	Mouse 0, 250, 500, 1000 ppm; gds 0-18 potassium dichromate 10 ♀/group	No deaths, or clinical or behavioral signs ↓ wt gain at 500 & 1000 ppm	No live litters or implantation sites at 1000 ppm ↓ fetuses/litter at 500 ppm ↑ pre-implantation loss at 500 ppm ↑ resorptions & post-implantation loss at 250 & 500 ppm

In most of these studies, treatment of females was completed before commencement of the mating period (Trivedi et al., 1989; Kanojia et al., 1996; Junaid et al., 1996b; Elbetieha and Al-Hamood, 1997; Kanojia et al., 1998). Nonetheless, adverse effects were seen in the offspring of previously-treated females mated with unexposed males. Observed fetal effects were similar to those observed with gestational exposures, as described in section C.2. (above), including: decreased viability of the conceptus (both pre- and post-implantation), decreased fetal weights and CRL, changes in placental weights (decreased or increased), and increased frequencies of external and skeletal anomalies. These effects were seen at Cr(VI) concentrations ranging from 250–750 ppm, with numbers and severity of effects increasing with increasing concentration.

Al-Hamood, et al. (1998), treated female mice throughout gestation and lactation, and examined the reproductive success of the F1 generation. These F1 offspring showed a significant lengthening of the estrous cycle, as well as decreases in the pregnancy index, implantation frequency, and viable fetuses at examination. The Murthy et al. (1996) study was designed to evaluate functional and histopathological effects of Cr(VI) on ovarian tissues. Estrous cycles were lengthened, ova counts decreased, and follicular damage was observed in treated females. While these effects were noted at Cr(VI)

total and hexavalent chromium measured in the breathing space of welders with personal monitors. As the table indicates, exposure to hexavalent chromium is not necessarily directly proportional to the level of chromium in the steel or the air space. Scheepers et al. (2008) found the median breathing space concentration of hexavalent chromium two times higher when welding mild steel compared to stainless steel, and comparable levels for mild and high alloy steels. On the other hand, Bonde and Christensen (1991) and Bonde et al. (1990a) reported higher Cr(VI) air concentrations during welding stainless steel compared to mild steel, although values for the groups overlap.

Table E1A. Chromium Exposures[a] of Welders Measured by Personal Air Monitoring

Type	N	Total chromium ($\mu g/m^3$)	Cr(VI) ($\mu g/m^3$)	Reference
Stainless Steel	30	11 (± 11 SD) [b]	3 (± 2 SD) [b]	Bonde and Christensen, 1991 (Denmark)
	7	14.8(11.4)	3.6[c] (± 2.8)	Bonde 1990a
	19	5.4 (2.3–387)	0.08 (<0.02–0.35)	Scheepers et al. 2008 (Netherlands)
High Alloy Steel	6	6.0 (0.59–6.83)	0.20 (<0.02–0.35)	Scheepers et al. 2008 (Netherlands)
Mild Steel	30	3 (± 8 SD)	1 (± 1 SD)	Bonde and Christensen, 1991 (Denmark)
	7 8	3.0 (±1.8 SD) 4.1(± 9.0 SD)	2.0 [c] (± 1.2 SD) 1.2 (± 1.2 SD)	Bonde 1990a
	28	1.3 (0.42–5.48)	0.23 (<0.02–2.38)	Scheepers et al. 2008 (Netherlands)

Abbreviations: SD, standard deviation; SS, stainless steel; TIG, tungsten inert gas.

[a]Values given are time weighted average median air concentrations in breathing space measured through a work shift by personal monitors. In parenthesis, ranges are given for the Scheepers et al. study and standard deviations (SD) for the Bond and Christensen study.

[b] TIG welding

[c] Mean rather than median value. The first value for mild steel for the Bonde (1990a) study is for MMA welding, the second is for MAG welding

Further insight into the potential exposure to Cr(VI) during welding can be garnered from biomonitoring. Determining indicators of Cr(VI) exposure is a challenge because of the difficulties in measuring low levels of Cr(VI) in biological samples and the relatively rapid reduction of Cr(VI) to Cr(III). Noting the influence of valence state on the absorption, transport and distribution of chromium, Minoia and Cavalleri (1988) examined the disposition of chromium in different blood compartments in workers exposed to Cr(VI) in order to identify a good marker of Cr(VI) exposure. They examined workers exposed to Cr(VI) and very high air levels of Cr(III). As shown in the table below, chromium in RBC was found to be a good marker of inhaled Cr(VI) exposure, consistent with earlier findings (Grey and Sterling, 1950; Lewalter et al. 1985). The data reflect the reduced absorption of Cr(III), its transport in serum and its inability to

Timing of Exposure Measurement

One cycle of spermatogenesis in men takes approximately 70 days, and it takes an additional seven days for sperm to transit and mature in the epididymis. Depending on the type(s) of germ cells Cr(VI) may target, it may take up to 12 weeks for the damage to recover, should it be reversible.

Air concentrations of chemicals in workplaces and levels of chemicals in urine or serum in occupational studies can be measured when the workers are examined for health effects. While concurrent exposure assessment is generally indicative of the overall exposure levels, it may not reflect the true exposure levels when the chemical targets germ cells in the testis, since it takes weeks for the cellular damage in germ cells to manifest as poor semen quality.

Semen quality

Jelnes and Knudsen. (1988). Stainless steel welding and semen quality.

Jelnes and Knudson (1988) performed a cross-sectional study of semen quality from a single "stainless steel industry" manufacturing plant in Denmark. The year that the cross-section was sampled was 1987. The total study group consisted of 226 age matched (±5 years) and smoking-habit matched male stainless steel welders and reference people (primarily nonwelders from the same plant). Blood, urine, and semen samples were collected. Blood samples were analyzed for chromium and nickel concentration, immunoglobulin G, and total protein. Urine was analyzed for mutagenic activity by the Ames test and for chromium and nickel concentration. Results of these analyses are not provided in the report. Semen was assessed by standard semen analysis. Two semen samples were delivered by 64% of those in the study group (145 men; 77 stainless steel welders and 68 non-welders). In cases where there were 2 semen samples, the second semen sample was used to improve clinical evaluation. The investigators compared the stainless steel welders' and non-welders' semen volume, sperm concentration (10^6/ml), percent live sperm, percent immotile sperm, and percent normal sperm. Statistical methods used to assess age and semen parameters include the Kruskal-Wallis test and two-sample t-test. No difference in semen parameters between the stainless steel welders and nonwelders was found. When the analysis was restricted to welders performing manual metal arc welding of stainless steel (n=20) and non-welders (n=11), again no differences in semen quality parameters were found. However, the authors note that "[e]xposure to metal dust is generally high also for nonwelders working at [these] plants" and they "might therefore be a suboptimal reference group."

Mortensen. (1988). Risk for reduced sperm quality among metal workers, with special reference to welders.

In 1988, Mortensen published a case-control study of stainless steel welding and reduced sperm quality in Denmark. Cases of reduced sperm quality were identified by

performing semen analysis for 3,119 men who had a connection with fertility problems and "had delivered a sample or samples of semen" to hospitals in four cities in the period 1981–1983. In 1984, a postal questionnaire was sent to men employed in the metal industry, certain other types of nonmetal industries, and other types of employment in which the factors suspected to influence sperm quality were not present. Men who received the postal questionnaire in three cities had not filled out a questionnaire at the time of semen sample delivery. The purpose of the questionnaire was to define possible influences of the work environment on sperm quality, and covered lifestyle questions such as smoking, alcohol consumption, use of medicines, and health status. Of the total 3,119 men included in the investigation, 81% (2,517) filled out the questionnaire satisfactorily (mean age 29.9 years). The number of questionnaires not returned was 602 (mean age of non-respondent men 31.0 years).

Cases (n=828) were defined as men meeting one or more of the following criteria: sperm concentration less then 20 million/ml, less than 50% of sperm cells motile, or less than 50% of sperm cells with normal morphology. In cases where more than one semen sample was collected, if one of two samples was normal quality and the other rated poor quality, the man was classified as a control. (1,480 men delivered one sample was and 1,037 men delivered 2 samples.) Men not defined as cases were used as the control group (n=1689). The OR was calculated with a log-likelihood measurement for an added risk of poor semen quality. Data were stratified by city and a Mantel-Haenszel OR was calculated. Confounders (age, smoking, alcohol, medicine, health status) were examined by means of a logistic regression analysis.

There were 27 welders classified as cases with reduced sperm quality, with 28 referents, for a total of 55 welders. The odds ratio was significant, calculated to be 2.0 (95% CI 1.16–3.45). The investigator further segregated the cases into stainless steel welders, and reported that risk of poor sperm quality was increased. This did not attain statistically significant when compared to nonwelders (OR=2.34, 95% CI 0.95–5.73), but the group size was smaller.

Bonde. (1990a). Semen quality and sex hormones among mild steel and stainless steel welders: a cross-sectional study.
Bonde. (1990b). Semen quality in welders before and after three weeks of non-exposure.
Bonde and Christensen. (1991). Chromium in biological samples from low-level exposed stainless steel and mild steel welders.
Bonde and Ernst. (1992a). Sex hormones and semen quality in welders exposed to hexavalent chromium.
Bonde. (1993). The risk of male subfecundity attributable to welding of metals: studies of semen quality, infertility, fertility, adverse pregnancy outcome and childhood malignancy.

Bonde (1990a; 1993) examined semen quality in a study of 135 men from six workplaces in Aalborg, Denmark that included welders (n=81), nonwelding metalworkers (internal referent group) and electricians (external referent group). Welders exposed to chromium

included both those engaged in welding of stainless steel (SS) (n=35) as well as those welding mild steel (MS).

Bonde (1990a) reported statistically significant effects on semen quality in SS welders in comparison to referents for decreased adjusted sperm count ($p<0.05$) and semen volume ($p<0.05$), percentage of subjects with immature sperm forms ($p<0.05$), the proportion of motile sperm ($p<0.05$), and increased percentages of sperm with poor (to no) motility ($p<0.01$). Decrements were also seen for some of these endpoints for MS welders, and for MS welders only, there were decreases in the proportion of sperm with normal morphology, and in linear penetration rates of sperm compared to referents.

The author hypothesized that discontinuance of exposure would improve sperm quality, and examined this question in a follow-up study of 53 workers (SS welders, n=18) from the main population who provided additional semen samples following a break in exposure (a three-week vacation) (Bonde, 1990b; Bonde, 1993). When after-vacation values (3, 5, and 8 weeks) were compared with before-vacation values, no improvement of semen quality was observed in the SS welders. In MS welders, a trend of improvement with time in post-vacation sperm concentration was observed ($p<0.05$) (Bonde, 1990b). The author noted that the lack of consistent evidence of improvement after the vacation might indicate that the effect of welding is not reversible within the short non-exposure period (Bonde, 1990b). However, as noted above, depending on the type of damage, this may have not been an adequate recovery period.

Bonde and Ernst (1992) obtained three sperm samples and, to determine levels of chromium, a spot urine sample and early in the shift a blood sample in a subgroup of the main population. Urine and sperm samples were obtained for 60 welders (SS welders, n=30) and 45 non-welding workers to evaluate exposure during a working day (post-shift, urine) and define baseline values (pre-shift, blood), the blood sample was obtained on a total of 86 workers. No correlation was found between the measurements of chromium in urine and blood and any of the sperm parameters (semen volume, sperm concentration, total sperm count, proportion of normal sperm forms, proportion of motile sperm, sperm penetration rate). There was a decrease in serum testosterone with increasing urinary chromium ($p=0.05$). Fifty-eight percent of the SS welders and 25% of the MS welders tested had urinary chromium levels in the highest tertile (>1.78 nmol $mmol^{-1}$ creatinine), as did 17% of the internal referent group; all of the electrician referent group had chromium levels in the lowest tertile (<1.07). Bonde (1993) notes a considerable nondifferential information bias in this study. The average exposure during the months preceding the provision of semen samples is, as noted by Bonde (1993), the exposure of interest, not the exposure during any particular day, and a welder may experience great variation in welding-fume exposure on consecutive days. Bonde (1993) also notes with regard to selection bias that the study is biased toward the null hypothesis. Volunteers who participated were more likely to have previous knowledge of reduced semen quality (obtained at, for example, an earlier infertility examination) (welders, OR=1.4, nonwelders, OR=4.2, both $p<0.05$) as well as a history of urogenital disorder (welders, OR=1.0, nonwelders, OR=3.2, both $p<0.05$); as seen by comparing the odds ratios, this selection bias was differential, occurring more often in nonwelding

participants. Also, since 17% of the internal referents population had chromium levels in the highest tertile, the comparisons of the welders to these individuals as "unexposed' is also called into question, although whole blood levels may be a better indicator of Cr(VI) exposure.

Hjollund et al. (1998a). Semen quality and sex hormones with reference to metal welding.

Hjollund et al. (1998a) examined semen quality in relation to welding exposure in the male partner in 430 Danish couples, including 130 couples in which the men were welders (29 current SS welders; others had historical exposure to SS welding). The couples were recruited nationally by letters sent via metal workers' unions and other trade unions. Only couples without previous reproductive experience who intended to discontinue contraception and attempt to become pregnant were included. Metal workers who had not welded in the previous three months were considered an internal referent group (71 couples). Excluding nonmetal working couples who reported doing some welding (n=24) left 205 couples who were nonmetal workers. Classification of exposure in this study of semen samples relied on self-reported data of a mainly dichotomous nature. At entry, each male provided one fresh semen sample, and a blood sample was drawn. During follow-up, monthly semen samples were collected and stored in the home freezer. The primary analyses were carried out on the fresh samples only. Analyses of fresh semen samples were based on 394 men.

No statistically significant differences were found in any exposure group in any semen quality measures (semen volume, sperm morphology, straight line and curvilinear velocity) compared to either of the two reference groups. Adjustment for potential confounding factors did not alter these results. The estimates of median sperm density changed to some extent for SS welders mostly due to adjustment for differences in distribution of sexual abstinence (mean 3.2 days for SS welders, 4 days for other welders). The authors reported that "analyses stratified on smoking resulted in practically identical values for smokers and nonsmokers." No differences were observed in urine concentrations of chromium, manganese, or nickel between welders and nonwelders or between pre-shift and post-shift urine samples (Hjollund et al., 1998a). The authors point to these results as some explanation of the findings; that is, the lack of an effect on semen quality could be due to generally low exposure of the study base. Despite the construction of several aggregated indices on the basis of information on welding method, type of welded metal, use of local exhaust ventilation, hours of welding, bystanding exposure, and employment in trades with well-known high exposures to welding fumes (e.g. shipyards), the authors report that they found no subpopulation of welders with elevated risks to any measure of semen quality.

Tielemans et al. (1999). Occupationally related exposures and reduced semen quality: a case-control study.

Tielemans et al. (1999) looked for associations between abnormal semen parameters and a wide variety of occupational exposures (organic solvents, metals, and pesticides) in a hypothesis generating case-control study in the Netherlands. Cases were men who were seen at two infertility clinics in the years 1995–1996 and who had abnormal sperm (based on concentration, motility, and morphology). Of the 1,536 men asked to participate, 75% (1,152) agreed. A total of 899 men were included in the study and delivered a semen sample after an abstinence period between 2 and 7 days. Semen evaluations were performed according to WHO procedures. The cases were divided into three severity-of-abnormality groups, with 692 cases under the least restrictive abnormality definition (sperm concentration $<20x10^6$/ml, <50% motile sperm, or <14% normal morphology), 267 cases under a more severe definition (sperm concentration $<5\ x10^6$/ml, <10% motile sperm, or <5% normal morphology), and 61 cases with azoospermia. Controls (n=207) were men who did not meet a case definition (sperm concentration $\geq 20x10^6$/ml, ≥50% motile sperm, or ≥14% normal morphology). All subjects provided background information (sociodemographic characteristics, lifestyle habits, infertility issues, time elapsed trying to conceive, and occupational history). Adjusted OR and 95% CI were calculated by logistic regression analysis. The investigators reported a nonsignificant decreased OR for the least restrictive case definition and welding of stainless steel of 0.53 (95% CI 0.21–1.37). When the cases were restricted to "primary infertile men", the odds ratio for stainless steel welding was slightly higher (OR=0.62, 95% CI 0.21–1.84). A subsample of cases (n=69) and controls (n=20) provided urine samples for analysis of chromium and other metal content in urine. For chromium, the mean urine level (μg/g creatinine) in cases (1.37) was approximately the same as in controls (1.29) (p=0.75).

Li et al. (2001). Effect of Cr(VI) exposure on sperm quality: human and animal studies. Li et al. (1999). Studies on male reproductive toxicity caused by hexavalent chromium. [Original in Chinese]

(The animal component of the study by Li et al. (2001) is described below in Section E.2.)

The cross-sectional study by Li et al. (2001) appeared to be reported first in Chinese by the same group of authors in 1999. All the major findings reported in 2001 appeared to be included in the 1999 report. However, the air monitoring data on air concentrations of Cr(VI) as reported in 1999 were not included in 2001 report. Therefore, the summary below is based mainly on the 2001 report, but the data on air concentrations of Cr(VI) are from the 1999 report.

In this cross-sectional study, Li et al. (2001) examined semen quality and sex hormones in the serum and seminal fluid of 21 male workers at an electroplating factory in China and corresponding parameters in 22 control workers from the same factory who were not exposed to chromium. The exposed and the control groups were aged 30.24±4.56 and

28.58±3.29 years (no statistically significant difference). However, no other data regarding the characteristics of the groups (smoking, drinking habits, reproductive or fertility history, etc.) were reported. The total number of exposed and unexposed male workers from which subjects were selected and the methods of selection were not stated. Exposure history (e.g., years of exposure to Cr(VI) or any other potential male reproductive hazards) was not reported.

The authors used generally accepted methods for their analyses of semen quality, levels of chromium (atomic absorbance spectrophotometer), zinc, lactate dehydrogenase (LDH), and lactate dehydrogenase C4 isoenzyme (LDH-x) in seminal plasma, and blood levels of chromium and hormones (FSH and LH). According to the 1999 report, air concentrations of Cr(VI) (expressed as CrO_3) during the survey period were 0.0012–0.8597 mg/m^3 (average 0.2351 mg/m^3) at the electroplating workshop and 0.0190–0.070 (average 0.0172 mg/m^3) at the preparation workshop. The authors did not report if the "average" was the mean or the median. The authors found that levels of chromium in the blood and seminal plasma from the exposed group were slightly higher than those from the control, but the differences were not statistically significant. The exposed group had significantly lower sperm counts (47.05±2.13 x 10^6/ml) and percentage of sperm with normal motility (69.71±0.93%), compared to the control group (88.96±3.40 x 10^6/ml and 81.92±0.41%, respectively). Sperm motility was not evaluated in this study. The levels of zinc, LDH, and LDH-x in seminal plasma were also significantly lower than those of the controls, indicative of increased state of oxidative stress. In addition, levels of FSH in the serum samples from the exposed group were significantly higher than those from the control, whereas no difference in the serum levels of LH between the two groups was reported. Interpretation of causality in the study is limited, however, by lack of data on potentially confounding variables (e.g., smoking) and lack of description of subject selection methods.

Danadevi et al. (2003). Semen quality of Indian welders occupationally exposed to nickel and chromium.

Danadevi and colleagues (2003) performed a cross-sectional study that compared semen quality between 57 welders (mean age 32.3 ± 4.4 years, range 21–41 years) and 57 non-welders (mean age 32.2 ± 4.7 years, range 23–40 years) in India, matched on age, "lifestyle" (including smoking and alcohol), and economic status. While the welders were said to be employed at a welding plant, the total numbers of welders from which the subjects were selected and the method of selection were not stated. The source of the non-welders and the year that the cross-section was selected were also not stated. Prior to the start of the study, each of the 114 subjects completed a questionnaire inquiring about age, smoking habits, duration of exposure, and usage of medicine. Medical histories and physical examinations were conducted, with emphasis on the genitourinary tract.

Twenty-eight male welders and 27 controls were selected randomly from the total number of subjects for blood metal analysis. Blood was sampled in the morning on the 4th day of the work week. Blood nickel and chromium concentrations were determined

using the ultra mass 700 inductively coupled mass spectrometer (ICP-MS). Weekly semen samples were collected after a 3-day period of abstinence from each participant for a period of 2 weeks. Semen was assessed according to WHO criteria. Liquefaction, volume, pH, viscosity, sperm agglutination, nonspecific aggregation, sperm count (10^6 sperm/ml), percentage of spermatozoa with motility of grades 1–3 and immotile sperm (grade 0), and concentration of white blood cells were examined. Log transformation was used for all semen parameters to improve normality of the data. The Chi-square test, Mann-Whitney U-test, and Spearman's correlation analyses were performed. The nonparametric Mann-Whitney test was used to analyze the difference in semen parameters between welders and controls.

While the welders were described simply as welders (as opposed to stainless steel welders), analysis of blood samples from a random sample of 28 welders and 27 non-welders showed much higher blood chromium levels among the welders (mean chromium levels 131.0 μg/l among welders versus 17.4 μg/l among non-welders). Many semen parameters were significantly different between welders and controls. The mean sperm count among welders was $14.5 \pm 24.0 \times 10^6$/ml compared with $62.8 \pm 43.7 \times 10^6$/ml among controls. The ejaculate volume was not significantly different. Sperm from welders also had significantly reduced progressive sperm motility, and vitality. A significant increase in abnormal morphology (head, mid-piece, and tail), and nonspecific aggregation were also noted among sperm from welders. Correlation analyses between blood chromium levels and semen parameters were conducted separately within welders and non-welders. Among welders, the blood chromium level was negatively correlated with sperm count, rapid linear progressive motility, and vitality. The negative correlations were statistically significant. A positive correlation between blood chromium and tail defects was also statistically significant. Among non-welders, blood chromium was significantly associated only with tail defects (positive correlation). The authors concluded that the results of the investigation indicated a significant reduction in semen quality of welders occupationally exposed to nickel and chromium. The strength of the findings, however, is tempered by lack of description of how welders and non-welders were selected for the study.

Kumar et al. (2005). Semen quality of industrial workers occupationally exposed to chromium.

In a cross-sectional study, Kumar et al. (2002) compared semen quality among 61 workers (mean age of 33.26 ± 6.97 years) at a chromate factory in India with semen quality among 15 pharmaceutical factory workers (mean age 24.2 ± 4.26 years). Lifestyle differences between groups included differences in smoking, drinking, and chewing habits; about 60% of the exposed group smoked whereas 20% of the controls smoked; 41% of exposed subjects drank alcohol whereas none drank alcohol among the controls; approximately 47% of exposed subjects chewed betel nut and tobacco whereas 53% of controls chewed the same. Detailed histories (including social, and medical histories) were collected from the subjects. Five to 6 ml blood samples and semen samples were collected from all subjects. Semen pH, semen liquefaction, sperm motility,

sperm viability, sperm concentration, sperm count, and sperm morphology were assessed. Statistical analyses used included the Student's t test and ANOVA to determine the significance between the two groups as well as to find out correlations between various parameters. Multiple regression was used for pooled data.

The total number of male workers at the chromate and pharmaceutical factories from which the subjects were enrolled, the methods of enrollment, and the year of enrollment were not stated. The investigators stated that they could not enroll a larger number of pharmaceutical factory workers "as the pharmaceutical workers were not persuaded to take part in the semen analysis." The investigators reported that the chromate exposed workers had statistically significantly more abnormal sperm morphology (53% of chromate workers versus 10% of pharmaceutical workers showing less than 30% normal forms). There were no significant differences, however, in other measures of semen quality (semen volume, liquefaction time, and pH, and sperm concentration, viability, and motility). The regression analyses conducted by the authors examined the effect of tobacco and alcohol use, and determined that these factors did not influence the sperm abnormality results, and so dropped them from the regression analysis. Interpretation of the study's findings is difficult because of possible strong selection bias in the control group, leading to potentially uncontrolled confounding. No information on the form of chromium (Cr(VI) or Cr(III)) to which the workers in the chromate factory were exposed was presented; however, chromate can be a Cr(VI) compound. Measurements of mean chromium in the blood in study participants were reported as 63.7 µg/l for chromate factory workers and 22.8 µg/l in the "unexposed" group. However, the authors also consider the rate of abnormal sperm morphology in those with nasal perforation (n=10) versus those without; the fact that some of these workers have been highly enough exposed to chromium to have perforated noses suggests that the exposure levels experienced by these workers may have been substantial. The IARC (1990) noted that nasal perforation was observed in two-thirds of chrome plating workers with inhalation exposure to peak chromium levels above 20 µg/m^3.

Infertility, Fecundability and Male-Mediated Spontaneous Abortion

Bonde et al. (1990c). Fertility among Danish male welders.

Bonde et al. (1990c) reported on a cohort study of fertility and birth outcome among welders in Denmark. The population of Danish males who had been employed for at least one year at 79 SS or MS manufacturing companies from April 1964 through December 1986 were identified from records of the Danish pension fund. Subjects with verified employment as a MS or SS welder or SS grinder, as well as non-welding and non-grinding production workers, were selected to constitute a cohort (n=10,059), with a subset of those born in 1945 or later (n=3702) the focus of the fertility study. Only 14% of these subjects had welded only SS. Analyses of SS welders reported by Bonde et al. (1990c) include all men who had welded SS, including those who also welded MS. Live born children fathered by cohort members from January 1968 through December 1986 were identified by record linkage to the Danish Central population registry. Fertility in

terms of birth rate was used as an indirect measure of fecundity. The parent-child relationship was verified for 99.4% of the children. Information on pregnancy outcome was obtained by record linkage to medical registers, including three different data sources.

Results of the fertility study in this nationwide cohort (Bonde et al., 1990c) indicated that the probability of a welder's wife having a child was statistically significantly reduced during years the man was at risk from any welding (MS or SS) exposure (RR=0.91, 95% CI 0.85 – 0.98). When the analyses were restricted to men who had welded SS (regardless of whether they also welded MS) and included consideration of exposure levels for SS welding, the decrease in fertility was slightly less and not statistically significant (OR=0.95, 95% CI 0.90 – 1.01). The results are based on comparison of at-risk years with years not at-risk in the same individuals. Bonde et al. (1990c) explain this approach by noting that "the probability of having a child in years not at risk from exposure was significantly greater [among men who had ever welded] than that of metalworkers who had never welded, even after adjustment" for a long list of variables. Bonde et al. (1990c) noted that a major problem in using fertility as a measure of fecundity in developed countries such as Denmark is the fact that most pregnancies are planned, and volitional factors cannot be accounted for in the analyses. The comparison of risk during years when welding took place versus year when it did not for the same individuals can only be valid if the effects of exposure are short-lived and do not influence fertility in years when exposure is not occurring. Since some other later studies suggest that the number of years spent welding may impact fecundability, the approach taken in this study may not be sufficiently sensitive.

Bonde et al. (1992b). Adverse pregnancy outcome and childhood malignancy with reference to paternal welding exposure.

In the nationwide cohort, Bonde et al. (1992b) reported that pregnancies at risk from paternal SS welding exposure were significantly more likely to be terminated by spontaneous abortion (adjusted OR=2.0, 95% CI 1.1 – 3.5). The risk estimate was higher for pregnancies at risk from MMA SS welding (OR=1.99, 95% CI 1.07 – 3.69) than for those at risk from TIG SS welding (OR=1.71, 95% CI 0.84 – 3.39), a finding the authors note indicates an exposure-response relationship (chi-square for trend 5.2, p=0.022). Because of the lack of specific dates for the spontaneous abortions and the potential exposure misclassification this could introduce, an analysis including only births with either exposure or nonexposure through all three years preceding the birth was carried out; risk of spontaneous abortion among wives of SS welders was still increased (OR=1.7, 95% CI 0.9 – 3.1), though no longer statistically significant. A slightly increased but not significant risk of pre-term delivery (defined as delivery more than three months pre-term) was also seen for children whose fathers were SS welders (OR=1.32, 95% CI 0.9 – 1.9). No evidence was seen for increased congenital malformation in children at risk from paternal welding (SS or MS). Factors impacting pregnancy outcomes were addressed by conducting multivariate regression analysis.

Bonde (1993). The risk of male subfecundity attributable to welding of metals: studies of semen quality, infertility, fertility, adverse pregnancy outcome and childhood malignancy.

From the original base population of men from six workplaces in Aalborg, Denmark, Bonde (1993) conducted a case-referent study among those who had completed a questionnaire, with cases identified as individuals reporting infertility (n=52) who were age-matched to others (n=208) who did not report infertility. The infertile group included those with welding exposure (n=40) and those without (n=12). Occurrence of infertility was evaluated in the questionnaire using answers to questions about difficulty conceiving based on at least two years of trying without success (no pregnancy). Comparison was also made to a larger group of men (n=563) who had reported fathering a child without delay of conception ("fertile" referents). Cases were classified as exposed if welding had taken place during the first year of infertility (± 1 year); age-matched referents were exposed if welding had been performed during the year of infertility of the corresponding case (± 1 year); fertile referents were exposed if they did welding in the year of childbirth or within the two years prior to childbirth. The rate of infertility among those with welding exposure was significantly elevated both when compared with age-matched referents (OR=2.2, 95% CI 1.1 – 4.6) as well as compared with the larger unmatched group of referents (OR=1.9, 95% CI 1.0 – 3.6), although the latter was a slightly lower ratio. No details were provided with respect to SS versus MS welding exposure for these study subjects.

Hjollund et al. (1995). Male-mediated risk of spontaneous abortion with reference to stainless steel welding.

Hjollund et al. (1995) conducted a re-assessment of spontaneous abortions in the same cohort examined by Bonde et al. (1992b). The source of information on spontaneous abortions in the cohort was different in the two studies: Hjollund et al. (1995) relied on Danish In-patient Hospital Register discharges with a diagnosis of spontaneous, induced or unspecified abortion, while Bonde et al. (1992b) analyses were based on spontaneous abortions reported to the midwife and subsequently recorded in the Medical Birth Register. Hjollund et al. (1995) note that, because their study is based on hospital records, earlier spontaneous abortions (which did not lead to hospitalization) may have been missed. The reports to the Medical Birth Register do not allow for consideration of the exact timing of paternal exposure relative to the outcome since the date of the abortion was unknown.

Hjollund et al. (1995) examined the discrepancies in reporting in the two systems for a subset of pregnancies, and found that more spontaneous abortions recorded in the Birth Register were missed by the Hospital Register than vice versa (14/82 compared to 7/82) (Hjollund et al., 1995). Nevertheless, because of their interest in better information on the timing of these events to determine potential for paternal exposure, these authors used the hospital-based data and found that the occurrence of spontaneous abortion was not increased in SS welders' wives (OR=0.78, 95% CI 0.55 – 1.1). The authors explain the

difference of the results of these two analyses by proposing that the earlier study's approach introduced bias by the method of assigning exposure status (whether or not the pregnancy was at risk from welding exposure), which Bonde et al. (1992b) had evaluated based on exposure status at the time of a subsequent birth. Hjollund et al. (1995) note that, for couples without children in this cohort, the male spouse was more likely to be welding SS than the male spouse of couples with one or more children, and this relation was independent of paternal age or birth year.

Hjollund et al. (1998b). A follow-up study of male exposure to welding and time to pregnancy.

The probability of conceiving in a given menstrual cycle (fecundability) was examined by Hjollund et al. (1998b) among 430 Danish couples, including 130 couples in which the men were welders; 29 of these were current SS welders, while others had historical exposure to SS welding. The couples were recruited nationally by letters sent via metal workers' unions and other trade unions. Only couples without previous reproductive experience who intended to discontinue contraception and attempt to become pregnant were included, and were followed for up to six menstrual cycles or until a pregnancy was recognized by a medical doctor. Questionnaires were used to collect self-reported information on occupational exposures including welding and various demographic, medical and other information. Metal workers who had not welded in the previous three months were considered an internal referent group (71 couples). Excluding nonmetal working couples who reported doing some welding (n=24) left 205 couples who were nonmetal workers. The menstrual cycle was the unit of observation, and cycles in which abstinence occurred during days 11 to 20 were excluded.

The association between risk factors and fecundability was estimated by a logistic regression model while controlling for a long list of potential confounding factors. The overall fecundability OR for couples with current exposure to male SS welding was 0.82 (95% CI 0.45 – 1.50) compared to nonwelding metal workers. The fecundability ratios for historical SS welding compared to either nonwelding metal workers or nonmetal workers showed an indication of decreasing fecundability with increasing years of SS welding (see table E3).

Table E3. Decreasing Fecundability[1] with Increasing Years of Welding Stainless Steel (from Hjollund et al., 1998b, Table 5)

Historical SS welding	Menstrual cycles at risk	OR (95% CI) compared to nonwelding metal workers [n=254 cycles]	OR (95% CI) compared to nonmetal workers [n=770 cycles]
≤1 year	71	1.51 (0.82 – 2.77)	1.73 (1.01 – 2.97)
2-5 years	73	0.77 (0.39 – 1.51)	1.00 (0.53 – 1.86)
6+ years	79	0.39 (0.18 – 0.86)*	0.56 (0.26 – 1.19)

* $p<0.05$

Abbreviation: SS, stainless steel

[1] Crude ratios, adjusted for cycle number but not for other potential confounders.

A significant interaction with male smoking was found when comparing welders to nonwelding metal workers (p = 0.04), whereas no significant interaction was found when comparing welders with the external reference group of nonmetal workers (p = 0.4); the authors subsequently presented results separately for smokers and nonsmokers. An internal analysis among ever welders (171 couples, 616 cycles) revealed a significant exposure duration-response relationship between years of welding and decreased fecundability among smokers (OR=0.76 per year welded SS, p=0.02); the decrease for nonsmokers was not statistically significant (OR =0.92 per year welded SS, no p-value reported). Fifteen percent of the welders stopped smoking simultaneously with discontinuation of contraception. Analysis of previous male smokers (2 years prior to enrollment) showed a persistent relationship of decreased fecundability for SS welding (OR=0.80 per year, p = 0.01).

Hjollund et al. (2000). Male mediated spontaneous abortion among spouses of stainless steel welders.

A study of male-mediated spontaneous abortion was conducted in the cohort of 430 Danish couples, including 130 couples in which the men were welders, 29 of whom were current SS welders, the same cohort reported on by Hjollund et al. (1998a, 1998b). The couples were recruited nationally by letters sent via metal workers' unions and other trade unions. Only couples without previous reproductive experience who intended to discontinue contraception and attempt to become pregnant were included, and were followed for up to six menstrual cycles or until a pregnancy was clinically recognized. Subclinical spontaneous abortions were detected in an analysis of urine samples during the time-to-pregnancy study via measurements of urinary human chorionic gonadotropin (hCG). Information on the outcome of pregnancies that were clinically diagnosed was obtained from 100% of the participants. Spontaneous abortion was defined as fetal loss occurring up to 28 weeks of gestation. Only the first pregnancy in any woman was included in the calculation of the rate ratios.

Of the 280 pregnancies detected, 71 ended in spontaneous abortions, of which 36 were clinically diagnosed and 35 were detected by hCG analysis and did not survive to a clinically detected pregnancy. The RR for spontaneous abortion of pregnancies with paternal SS welding exposure was significantly increased relative to pregnancies without welding exposure (RR=2.6, 95% CI 1.2 – 5.5). Adjustment for a long list of potential confounding factors did not alter the risk, according to the authors. The risk was still significant in separate analyses of early pregnancy loss (RR=3.0, 95% CI 1.1 – 8.0) and loss of clinically recognized pregnancies (RR=3.2, 95% CI 1.1 – 9.8). All spontaneous abortions in spouses of SS welders took place before the 10^{th} gestational week. Risk of pregnancy loss increased as years of SS welding increased (1 to 5 years, RR=1.2, 95% CI 0.4 – 3.3; >5 years, RR=2.6, 95% CI 1.1 – 6.1). The authors note that exposure information was collected prior to pregnancy outcome for all pregnancies, and timing of pregnancy recognition was not different among exposed and unexposed subjects. Hjollund et al. (2000) state that their findings "indicate an increased risk of early

spontaneous abortion for women whose partners are engaged in stainless steel welding during the cycle in which woman conceived."

Given the lack of findings of effects of welding on semen quality in males included in this study population (reported in Hjollund et al., 1998a), the authors speculate that the mechanism responsible may not be reflected in the traditional parameters of semen quality. Hjollund et al. (2000) refer to studies of toxicokinetics that indicate a slow elimination of chromium from some body compartments, and note that their data "suggest that long-term exposure may be as relevant a risk indicator as current exposure."

Hjollund et al. (2005). Spontaneous abortion in IVF couples--a role of male welding exposure.

Hjollund and colleagues (2005) examined spontaneous abortion risk and paternal welding exposure, including stainless steel (SS) welding, among *in vitro* fertilization (IVF) clinic couples in Denmark. The couples were identified through the Danish In Vitro Fertilization Registry, and the study included couples for whom embryo transfers occurred during the years 1996–2000. Pregnancy was defined as a positive hCG analysis. Spontaneous abortion was defined as a clinically recognized miscarriage through the first 28 weeks of pregnancy or an hCG defined pregnancy that did not end in a pregnancy outcome as determined by linkage to the Danish Birth Registry and Danish Hospital Registry. A postal questionnaire solicited information on reproductive history, education, job, occupational exposures, smoking, alcohol, and other lifestyle issues. After one reminder, 4,007 useable questionnaires were received (68% response). Men who indicated that they were welders (n=512) were sent a second questionnaire with greater specificity about the types of welding and when the welding was performed. A total of 91 pregnancies with paternal SS welding exposure were included in the study. The majority (85%) of these men welding SS did so less than one hour per day. The authors also note that most participants used protective measures during welding. The spontaneous abortion rate was 28.4% among 2,925 non-exposed (no paternal welding) pregnancies, while that among pregnancies with SS exposure was 17.6%. Risk ratios were not elevated for SS welding (RR=0.59, 95% CI 0.36 – 0.98) nor did analyses by years of SS welding show any indication of increased risk (<1 year, RR=0.93, 95% CI 0.48-1.79; 1–5 years, 0.94, 0.55–1.60; 6+ years, 0.68, 0.38 – 1.25). The investigators concluded that their study provided no evidence of excess risk of spontaneous abortion from paternal welding, but they emphasized that findings based on IVF pregnancies may not apply to other pregnancies. In particular, they note that if more than one embryo is successfully implanted, a spontaneous abortion is only registered in the case of death of all fetuses; in more than 80% of the cases in this study, more than one embryo was implanted.

E.2. Animal male reproductive toxicity studies

Available data on the potential male reproductive toxicity of Cr(VI) in experimental animals includes four reproductive studies performed by the oral route (drinking water) in monkeys (Aruldhas et al., 2004; Aruldhas et al., 2005; Aruldhas et al., 2006; Subramanian et al., 2006). Of seven studies conducted in rats, one provided Cr(VI) in drinking water (Bataineh et al., 1997), two were performed by feed or gavage (Chowdhury and Mitra, 1995; Li et al., 2001), and the remaining four by i.p. injection (Ernst, 1990; Saxena et al., 1990a; Murthy et al., 1991; Ernst and Bonde, 1992). Three mouse studies were conducted by the oral route of exposure, one by drinking water (Elbetieha and Al-Hamood, 1997), and two by feed (Zahid et al., 1990; Al-Hamood et al., 1998). Of two studies in rabbits, one was conducted by gavage (Yousef et al., 2006) and the other by i.p. injection (Behari et al., 1978).

Non-human primate, oral

*Subramanian et al. (2006). Reproductive toxicity of chromium in adult bonnet monkeys (*Macaca radiata *Geoffrey) Reversible oxidative stress in the semen.*

Adult male bonnet monkeys (*Macaca radiata* Geoffrey) were used in a study to test the hypothesis that oxidative stress mediates chromium-induced toxicity. Animals were divided into groups of three, and given Cr(VI) in the form of potassium dichromate in drinking water for six months at concentrations of 0, 50, 100, 200, or 400 ppm. A maximum concentration of 400 ppm was chosen as higher concentrations had previously been shown to result in death within three months. Additional groups of monkeys were treated simultaneously with 400 ppm Cr(VI) plus vitamin C at 0.5, 1.0, or 2.0 g/L for six months. A final group of monkeys was treated with 400 ppm Cr(VI) for six months, followed by a six month untreated period, to see if recovery from Cr(VI) toxicity would occur. Over the course of the study, blood and semen samples were collected on a monthly basis. Sperm counts and sperm motility were determined prior to separation of the sperm and seminal plasma. Seminal plasma and lysed sperm were then used in assays for enzyme activity and product concentration.

At 50 ppm Cr(VI), sperm counts were unaffected over the treatment period. At all other Cr(VI) concentrations, sperm counts were significantly decreased ($p < 0.05$) in a dose and duration-dependent manner. The 400 ppm group showed a significant decrease starting at two months on treatment. The 200 ppm group showed a significant decrease starting at three months on treatment, and the 100 ppm group starting at four months on treatment. For the group treated for six months with 400 ppm Cr(VI), and then withdrawn from treatment for another six months, sperm counts returned to control levels after three months of withdrawal from treatment. All three concentrations of Vitamin C prevented the sperm-depleting effects of exposure to 400 ppm Cr(VI). Sperm motility data followed exactly the same pattern of as sperm counts, for all time points and treatments.

Superoxide dismutase (SOD) activity in seminal plasma and sperm was not affected in the 50 ppm Cr(VI) group. At 400 ppm, in contrast, significant decreases ($P < 0.05$)

relative to controls were seen from one month of treatment on. At 200 and 100 ppm, significant decreases in SOD were first observed at three and five months of treatment, respectively ($p < 0.05$ in all cases). After six months exposure to 400 ppm Cr(VI), followed by additional time without treatment, seminal plasma and sperm SOD levels returned to control values by four months of recovery time. Simultaneous Vitamin C treatment (all three concentrations) maintained SOD activity in animals given 400 ppm Cr(VI).

Catalase activity in seminal plasma and sperm was not affected in the 50 ppm Cr(VI) group. In seminal plasma, catalase showed significant decreases ($p < 0.05$) starting at two months in the 400 ppm group, at three months in the 200 ppm group, and at five months in the 100 ppm Cr(VI) group. In spermatozoa, the pattern of decreases was similar, excepting that significant changes ($p < 0.05$) were first observed in the 400 ppm group after one month of treatment. In both seminal plasma and sperm, catalase activity return to control levels after four months of recovery from six months exposure to 400 ppm. Simultaneous Vitamin C treatment (all three concentrations) maintained catalase activity in animals given 400 ppm Cr(VI).

Levels of reduced glutathione in seminal plasma and sperm were not significantly affected by six months of exposure to 50 or 100 ppm Cr(VI). In seminal plasma, decreases ($p < 0.05$) in reduced glutathione levels were observed starting at one month of exposure for the 400 ppm group, and at three months of exposure for the 200 ppm group. Six months of exposure to 400 ppm, followed by five months of recovery, saw levels of reduced glutathione in seminal plasma returned to normal. In sperm, significant decreases in reduced glutathione levels ($p < 0.05$) were first seen at two months of exposure to 400 ppm, but at one month of exposure to 200 ppm Cr(VI). Reduced glutathione levels returned to control values following four months of recovery from six months exposure to 400 ppm Cr(VI). Vitamin C (all three concentrations) prevented the decreases in reduced glutathione otherwise seen with 400 ppm Cr(VI) for both seminal plasma and sperm.

Hydrogen peroxide (H_2O_2) concentration in either seminal plasma or sperm was not affected by 50 ppm Cr(VI). In seminal plasma, Cr(VI) concentrations of 400 or 200 ppm were found to result in significantly increased ($p < 0.05$) levels of H_2O_2, starting after one month of treatment. Increases were seen in H_2O_2 levels in the 100 ppm group after three months of treatment ($p < 0.05$). It took four months of recovery following six months of exposure to 400 ppm Cr(VI) for H_2O_2 to be restored to control levels. After three months of exposure to Cr(VI), H_2O_2 levels in sperm were significantly increased ($p < 0.05$) relative to controls for the 400, 200, and 100 ppm groups. All three concentrations of Vitamin C were effective at masking the effects of Cr(VI) on H_2O_2 in seminal plasma or sperm.

Plasma chromium concentrations were significantly increased in all four treated groups, in a concentration-dependent manner, by the end of one month of treatment ($p < 0.05$). Over a six-month recovery period following six months of exposure to 400 ppm Cr(VI), plasma chromium levels dropped but remained significantly ($p < 0.05$) above control

levels. All three concentrations of Vitamin C reduced plasma chromium concentrations in treated (400 ppm) animals to some extent, but did not restore control levels.

The authors concluded that chronic Cr(VI) exposure resulted in male reproductive toxicity, which increased in severity with increasing dose and duration. Their data were taken to support the hypothesis that chronic Cr(VI) exposure caused a reversible oxidative stress in the seminal plasma and sperm, leading to sperm death and reduced mobility of live sperm.

*Aruldhas et al. (2006). In vivo spermatotoxic effect of chromium as reflected in the epididymal epithelial principal cells, basal cells, and intraepithelial macrophages of a nonhuman primate (*Macaca radiata *Geoffroy).*

Adult, male macaque monkeys (*Macaca radiata*) were divided into four groups of three each. For a period of 180 days, the animals were given drinking water containing potassium dichromate to Cr(VI) concentrations of 0, 100, 200, or 400 ppm. There is no mention of collecting data on body weights or clinical symptoms of toxicity. At the end of the treatment period, the animals were anesthetized for removal of testes and epididymides. After healing from surgery, the animals were released to a reserve forest, according to guidelines specified by the Indian government. Thin sections of epididymides were prepared for transmission electron microscopy.

As compared to sections from control monkeys, epididymal tissues of treated monkeys featured striking accumulation of sperm-derived lipofuscian (LF) material in the principal cells, basal cells, and intraepithelial macrophages of the epithelium. Principal cells apparently phagocytosed dead sperm from the lumen, partially processing them into LF material. Liopfuscian material was then picked up by the basal and intraepithelial macrophages and processed further. Although the authors do not present dose-response data, they state that "the responses increased with the dose of Cr(VI)...."

The authors conclude that spermatotoxicity must be among the adverse effects of Cr(VI) exposure, as live sperm would not have been phagocytosed. They propose that damage to the epididymal luminal spermatozoa caused them to be taken up and phagocytosed by principal cells, followed by increases in the frequencies of basal cells and intraepithelial macrophages with densely accumulated LF inclusions -- the LF material representing the residue of lysosomal hydrolysis.

*Aruldhas et al. (2005). Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (*Macaca radiata *Geoffroy).*

Adult male bonnet monkeys (*Macaca radiata*) were divided into four groups of six animals each. Each of three treatment groups were provided with drinking water containing Cr(VI) (potassium dichromate) at concentrations of 100, 200, or 400 ppm, *ad*

libitum for 180 days. The fourth group was given plain water. The Cr(VI) concentrations used in this study were selected on the basis of pilot experiments which had demonstrated effects on spermatogenesis in the absence of severe systemic toxicity. At the end of the 180-day dosing period, testes were surgically removed from half of the animals. Testes were surgically removed from the remaining animals after a further 180 days without treatment. Blood samples were taken at regular intervals throughout the study. Right testes were fixed and prepared for light and transmission electron microscopy (TEM), while the left testes were prepared for biochemical analysis.

Plasma chromium levels at 24 hours following the last day of treatment were increased up to ten-fold in treated monkeys ($p < 0.05$ at all concentrations). Chromium levels declined to control values after 180 days recovery following treatment. While absolute testes weights were not appreciably affected by treatment, relative testes weights showed significant ($p < 0.05$) decreases at all Cr(VI) concentrations when evaluated directly following the end of treatment. These weights returned to normal, however, following 180 days of recovery from treatment.

At the light microscopic level, seminiferous tubules of control monkeys were reported to be normal. The seminiferous tubules of treated monkeys, on the other hand, were described as disorganized, with tubules of decreasing diameter with increasing Cr(VI) concentrations. The most common features among experimental groups were depletion of germ cells and hyperplasia of Leydig cells. Additional observations included: absence of spermatids in some tubules, Sertoli cell fibrosis, vacuoles surrounding spermatids still adherent to the epithelium, multinucleate giant cells in the adluminal compartment, lumen filled with prematurely released germ cells and cell debris, and abnormal appearance of chromatin in postzygotene spermatocytes. In the animals allowed six months of recovery following chromium exposure, appearance of the testes at the light level was essentially comparable to controls.

Transmission electron microscopy revealed treatment-related changes in testicular structure including: granulated chromatin and vacuolation in spermatids, fragmented chromatin and swollen mitochondria with collapsed christae in pachytene spermatocytes, and macrophages containing phagocytosed sperm and dense inclusions in Sertoli cells. Preleptotene spermatocytes and spermatogonia in the basal compartment were not affected.

The specific activities of testicular superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase, considered to indicate the status of oxidative stress in the testis, were all significantly decreased ($p < 0.05$) relative to controls after 180 days on any of the test concentrations of Cr(VI). The specific activity of testicular γ-glutamyl-transpeptidase was significantly ($p < 0.05$) different from controls only at the high concentration of 400 ppm Cr(VI). Glutathione-S-transferase activity, as well as the concentrations of reduced glutathione, hydroxyl radical, and hydrogen peroxide were all significantly *increased* after 180 days in any of the Cr(VI) test groups. Testicular concentrations of vitamins C, A, and E were all significantly lower ($p < 0.05$) in all Cr(VI)-exposed groups than in controls.

The authors concluded that Cr(VI) disrupts spermatogenesis by inducing free-radical toxicity. They further suggest that supplementation with antioxidant vitamins might be of therapeutic benefit.

Aruldhas et al. (2004). Microcanalization in the epididymis to overcome ductal obstruction caused by chronic exposure to chromium - a study in the mature bonnet monkey (Macaca radiata Geoffroy*).*

Adult male bonnet monkeys (*Macaca radiata*) were divided into four groups of three animals each. Potassium dichromate was added to drinking water to provide Cr(VI) concentrations of 0, 100, 200, or 400 ppm. At the end of a 180-day experimental period, the testes and epididymides were surgically removed from animals under sodium pentathol anesthesia. Tissues were prepared for light microscopy and TEM.

The authors provide detailed descriptions of two types of "microcanals" observed in the epididymal epithelium of treated animals. They hypothesize that the first type of microcanal provides passage for spermatozoa to bypass the blocked main duct. In contrast, the second type of microcanal was proposed as a means by which spermatozoa reaching the core of the epithelium are sequestered, as a mechanism to avoid an autoimmune response. Although these effects were not quantified, the authors' believed that the incidence and severity of microcanalization increased with increasing Cr(VI) concentration, with the 400 ppm group being the most affected. They interpreted their findings as indicative of Cr(VI)-induced obstruction of the distal portion of the cauda epididymis.

Rat, oral (drinking water)

Bataineh et al. (1997). Effects of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat.

Forty adult male Sprague-Dawley rats, each weighing approximately 300 g, were randomly divided into three groups: 13 animals served as controls, 15 animals were given chromium chloride (Cr(III)) in drinking water, and 12 animals were given potassium dichromate (Cr(VI)) in drinking water. Both compounds were added to water in a concentration of 1000 ppm for 12 weeks. Mating behavior, aggressive behavior, and fertility were evaluated during the treatment period. It is not clear from the paper exactly when behavioral observations were made, but the fertility tests are stated to have been conducted at the end of 12 weeks of treatment. At completion of the study, the animals were sacrificed and body weights and weights of reproductive organs were recorded.

In sexual behavior studies, males were placed in the observation cages five minutes before a female in estrous was introduced. Numbers of mounts were significantly decreased ($p < 0.001$), while ejaculatory latency and post-ejaculatory intervals were both

significantly increased ($p < 0.001$) in Cr(VI)-treated animals. The Cr(VI)-exposed group also had a significantly lower percentage of males ejaculating ($p < 0.005$). Hexavalent chromium-exposed males did not differ significantly from controls in time to first mount, intromission latency, or number of ejaculations.

For aggression testing, a stud male was placed in an observation cage for 10 days. A test animal was then added, and behaviors recorded over a five-minute period. Significant decreases compared to controls were seen in Cr(VI)-exposed animals for all parameters recorded. Lateralizations by stud male, boxing bouts with stud male, and fights with stud male were all decreased at the $p < 0.001$ level of significance. Ventral presenting was decreased with a significance level of $p < 0.05$.

In fertility studies, each male was caged with two females for a period of 10 days, allowing for two complete estrous cycles to occur. No significant effects of Cr(VI) exposure were reported for the numbers of pregnant females, or the numbers of implantations or viable fetuses. The total number of resorptions was increased from 0 in controls to 9 for the Cr(VI)-treated group, but this appears to be a total for the group, rather than a litter average, and either was not statistically significant or no statistical analysis was performed.

Body weights and absolute weights of testes, seminal vesicles, and preputial glands were all significantly reduced ($p < 0.001$) in Cr(VI)-exposed animals. Relative testes weights were also significantly reduced in treated animals ($p < 0.05$). Relative seminal vesicle and preputial gland weights did not differ between Cr(VI)-treated animals and controls.

The authors interpret the results of their behavioral studies as indicating an effect of chromium on testicular testosterone production. Fertility, under the conditions tested in this study, was not affected.

Rat, oral (feed or gavage)

Li et al. (2001). Effect of Cr(VI) exposure on sperm quality: human and animal studies.

In a study that also included an epidemiological component discussed above in Section E.1.1., sixty-day-old male Wistar rats were divided into three groups of N = 8–11 per group. The animals were given CrO_3 at doses of 0, 10, or 20 mg/kg-day by "oral feeding" for six days. It is not clear from the paper whether treatment was by gavage, or by mixing the test compound into the animals' feed. At six weeks following treatment, the rats were sacrificed and testes and epididymides removed for sperm evaluations and histological evaluation of testicular tissues.

Epididymal sperm counts were significantly reduced ($p < 0.05$) at both doses of CrO_3, in an apparently dose-dependant manner. The frequencies of abnormal sperm were significantly increased in both treated groups ($p < 0.01$), again in an apparent dose-dependent manner. See Table E4 below for details.

Table E4. Epididymal sperm counts and frequencies of abnormal sperm in Wistar rats exposed to Cr(VI) via drinking water by Li et al. (2001)

Cr(VI) dose	0 mg/kg-day	10 mg/kg-day	20 mg/kg-day
No. rats	9	8	11
Sperm count × 10^6/g epididymis	87.40 ± 3.85	21.4 ± 1.2*	17.48 ± 1.04*
% abnormal sperm	2.75 ± 0.06	6.68 ± 0.32**	7.6 ± 0.15**

* $p < 0.05$; ** $p < 0.01$

Histopathology revealed a Cr(VI)-associated decrease in the diameter of seminiferous tubules, as well as disruption of the germ cell arrangement near the seminiferous tubules.

Chowdhury and Mitra. (1995). Spermatogenic and steroidogenic impairment after chromium treatment in rats.

Mature male Charles Foster rats, weighing approximately 150 g, were divided into four groups of ten animals each. Treatment consisted of gavage dosing with sodium dichromate at doses of 0, 20, 40, or 60 mg/kg-day for a period of 90 days. At the end of the treatment period, the animals were sacrificed and their testes removed for evaluation.

Final body weights and percent weight gain appeared to be lower than controls for the 40 and 60 mg/kg-day Cr(VI) groups, though statistics were not presented for these endpoints. Mean testis weights were significantly lower than controls at both 40 ($p<0.05$) and 60 ($p<0.001$) mg/kg-day. Testicular protein content was found to be significantly reduced compared to controls in all treated groups ($p<0.05$ at 20 mg/kg-day, $p<0.001$ at 40 and 60 mg/kg-day); reduction occurred in a dose-dependent manner.

Table E5. Male reproductive toxicity data in Charles Foster rats exposed to Cr(VI) via gavage by Chowdhury and Mitra (1995)

Parameter/Dose (mg/kg-day)	0	20	40	60
Wt. gain (%)	102.4	100.38	43.40	42.32
Testes wt. (g)	1.480 $\pm$ 0.93	1.440 $\pm$ 0.03	1.07 $\pm$ 0.62*	0.962 $\pm$ 0.018***
Testicular protein (mg/g tissue)	121.30 $\pm$ 0.935	110.94 $\pm$ 0.121*	75.20 $\pm$ 1.020***	65.0 $\pm$ 2.181***
Testicular DNA (mg/g tissue)	13.1 $\pm$ 0.155	13.3 $\pm$ 0.267	9.30 $\pm$ 0.243***	7.25 $\pm$ 0.220***
Testicular RNA (mg/g tissue)	7.9 $\pm$ 0.183	7.2 $\pm$ 0.153	5.1 $\pm$ 0.182***	5.0 $\pm$ 0.138***

*$p < 0.05$; ***$p < 0.001$

Testicular DNA and RNA contents were significantly lower than controls at 40 and 60 mg/kg-day ($p<0.001$ for both endpoints at both doses). Seminiferous tubule diameter was significantly reduced at both 40 and 60 mg/kg-day ($p<0.001$ at both doses). Leydig cell populations were significantly reduced at both 40 and 60 mg/kg-day ($p<0.05$ for both doses). Degenerative changes in Leydig cells, and reduced Leydig cell nuclear diameter were observed at 40 and 60 mg Cr(VI)/kg-day.

When spermatogenic cell counts were expressed as a ratio of eight Sertoli cells, the following relationships to treatment were found:

- There were no differences among groups for spermatogonia counts
- Resting spermatocyte counts were significantly reduced relative to controls at the high dose of 60 mg/kg-day ($p<0.05$)
- Pachytene spermatocyte counts were significantly reduced at 40 and 60 mg/kg-day ($p<0.05$ for both doses)
- Stage-7 spermatid counts were significantly reduced at both 40 and 60 mg/kg-day ($p<0.05$ and $p<0.001$, respectively)

Testicular cholesterol was significantly *increased* at 40 and 60 mg Cr(VI)/kg-day ($p<0.05$ for both doses). Conversely, testicular levels of succinic dehydrogenase and $3\beta\Delta^5$-hydroxy steroid dehydrogenase with NADPH ($3\beta\Delta^5$-HSH) were significantly reduced relative to controls at 40 and 60 mg/kg-day ($p<0.05$ or less for both endpoints at both doses). Only $3\beta\Delta^5$-HSH was also significantly reduced at 20 mg/kg-day ($p<0.05$). Serum testosterone levels were significantly reduced at all three dose levels ($p<0.05$ for 20 mg/kg-day, and $p<0.001$ for both 40 and 60 mg/kg-day).

The authors concluded that the reductions in testicular DNA and RNA contents of Cr(VI)-treated animals indicated low cellular turnover, which was consistent with reductions in seminiferous tubule diameter observed in these same animals. The increase in testicular cholesterol also observed at the two higher doses of Cr(VI) was taken as indicative of "nonutilization of cholesterol towards testosterone synthesis in testicular tissue." Impaired testosterone synthesis was also indicated by the findings of reduced testicular $3\beta\Delta^5$-HSH (a key enzyme for testosterone biosynthesis), as well as the reductions in serum testosterone in treated animals.

Rat, i.p.

Ernst and Bonde. (1992). Sex hormones and epididymal sperm parameters in rats following sub-chronic treatment with hexavalent chromium.

Adult male Wistar rats (100–120 days old) were randomly assigned to one of four groups: two control groups, and two treated groups. Each group consisted of ten animals. Both treated groups were given Cr(VI) as sodium chromate by i.p. injection, at a dose of a dose of 0.05 mg/kg (reported in the paper as 0.05 mg kg^{-1}), daily, five days per week, for a period of eight weeks. Controls were given injections of an equal volume of saline.

Immediately following the end of the exposure period, one treated group and one control group were sacrificed for investigation. The other control and treated groups were held for an additional eight-week recovery period before examination. Blood samples and right epididymides were obtained from each animal. Epididymal spermatozoa were flushed for microscopic observation.

Over the course of the study, animals were observed for clinical signs of toxicity, and none were found. Body weights of all animals increased during the study, but there were no significant differences between treated and untreated rats.

Epididymal sperm counts were reduced in Cr(VI)-exposed animals examined just after cessation of the treatment period ($600 \pm 34 \times 10^{-6}$, as compared to $623 \pm 24 \times 10^{-6}$ for corresponding controls), but the difference was not statistically significant. No difference was found in sperm counts between control and treated animals following an eight week recovery period. The frequency of abnormal sperm was not altered in any of the four groups. The percentage of motile sperm was significantly ($p<0.001$) affected only in treated animals examined immediately following the end of treatment ($30 \pm 6\%$, as compared to $63.5 \pm 5\%$ for corresponding controls).

For animals examined directly at the end of the treatment period, testosterone levels were significantly decreased ($p<0.05$), while FSH and LH were significantly increased ($p<0.001$ and $p<0.05$, respectively) in Cr(VI)-exposed animals. For animals treated and then allowed to recover for a further eight-weeks, only LH levels were affected, showing a significant decrease compared to corresponding controls ($p<0.05$).

Murthy et al. (1991). Ultrastructural observations in testicular tissue of chromium-treated rats.

Adult male albino rats of the Druckery strain (90 days old) were divided into two groups: a control group, and a group given 2 mg Cr(VI) (in the form of potassium dichromate)/kg bw-day by i.p. injection for 15 days. Body weight, as well as feed and water consumption, were recorded on alternate days. Five animals from each group were sacrificed on the 16th experimental day; blood samples and testes were taken for chromium-content determinations. Epididymides were also taken from these animals for sperm counts and sperm motility studies. Three additional animals from each group were anesthetized, and perfused with fixative containing 2% lanthanum nitrate, as a tracer molecule to test the integrity of the blood-testis barrier. The testes were then removed and prepared for light or electron microscopy.

Body weight, and feed and water consumption did not differ between treated and control groups. Neither sperm counts nor sperm motility showed significant differences between the two groups. Blood and testicular chromium levels, however, did show significant increases in treated animals ($p < 0.01$).

At the light microscopy level, testes of both control and treated animals were described as having "an orderly arrangement of germ cells and cellular strands of interstitial tissue containing normal Leydig cells under the light microscope." Lanthanum distribution in control testes "was limited to the Sertoli cell junctions in the basal compartment of the seminiferous epithelium." In testes from treated animals, lanthanum appeared more widely "between Sertoli cells and also in the Sertoli-Sertoli cell junctions of the adluminal compartment of the seminiferous tubule, restricted to the primary spermatocyte level." Other anomalies in the testicular tissues of Cr(VI)-treated animals included: focal tubular damage, vacuolization of cytoplasm and disappearance of mitochondrial cristae in seminiferous epithelium of the damaged tubules. Vacuolated cytoplasm was also seen in Sertoli cells of affected tubules. Damage to mitochondrial sheaths of spermatid tail midpieces were also observed in the damaged tubules of treated testes.

The authors conclude that penetration of the lanthanum tracer molecules beyond the basal compartment in Cr(VI)-treated rats indicates Cr(VI)-induced damage to Sertoli cell membrane properties and the blood-testis barrier.

Saxena et al. (1990b). Effect of hexavalent chromium on testicular maturation in the rat.

Sixty weaned male albino rats, weighing 40 to 60 g, were divided into four groups of 15. Each group was given i.p. injections of 0, 1, 2, or 3 mg Cr(VI) (as potassium dichromate)/kg-day until the animals reached 90 days of age. Body weights were recorded on alternate days. Six animals from each group were sacrificed at 55 days of age, and six more at 90 days of age. Blood samples and testes were taken from all animals; epididymides were also removed from the 90-day old animals.

Animals given 1 mg Cr(VI)/kg-day showed no clinical symptoms during treatment, and all animals survived. At the higher doses of 2 and 3 mg Cr(VI)/kg-day, two and three rats died, respectively. The causes of these deaths could not be determined by necropsy. Body weights of animals in the two higher dose groups were markedly lower than control measures from the first week of treatment onwards. No statistical analysis of body weight data was presented. Absolute testes weights were significantly reduced ($p < 0.05$) compared to controls at both 55 and 90 days in the groups given doses of 2 or 3 mg Cr(VI)/kg-day. At 90 days there was an obvious and statistically significant ($p < 0.05$) difference between these two doses, with the lower testes weights associated with the higher dose. No significant differences were found among groups for testes weights relative to body weight, at either time point for any dose.

At 55 days of age, histological examination of testes did not reveal any differences among groups. Tubular and interstitial tissues were found to have normal appearance in all testes examined. By age 90 days, pathological changes were observed in all treated groups, increasing in severity with increasing dose. Observations included: disturbed spermatogenesis in the form of multinucleated giant cells and pyknotic nuclei, shrunken and damaged tubules that were devoid of sperm in their lumens, focal interstitial edema,

atrophied Leydig cells, and decreases in the numbers of late-stage spermatids and germ cell numbers at stage VII.

Histological examination of testicular tissues at age 90 days revealed a general decrease in seminiferous tubule diameter, which reached strong significance at the high dose of 3 mg Cr(VI)/kg-day ($p < 0.05$ compared to controls or other dose groups). A similar pattern was seen for reduction of Leydig cell nucleus diameter, although a significant decrease ($p < 0.05$) from controls was also seen at 2 mg Cr(VI)/kg-day.

Epididymal sperm counts and sperm motility at 90 days of age both decreased with increasing dose of Cr(VI). Sperm count at the low dose was reduced by approximately 16%, compared to that of the controls, though the reduction was not statistically significant. At the mid dose, these differences were significantly different from controls ($p< 0.05$); at the high dose, both endpoints were significantly lower than those for other dose groups as well as controls ($p < 0.05$ in all cases). See table E6 below.

Table E6. Epididymal sperm counts and sperm motility at 90 days in rats given ip injections of Cr(VI) for 90 days by Saxena et al. (1990b)

Cr(VI)/kg-day	**0**	**1**	**2**	**3**
Number rats	6	6	6	6
Sperm count (million/ml)	45.78 $\pm$ 3.3	34.23 $\pm$ 4.27	24.01 $\pm$ 3.82*	16.52 $\pm$ 1.61*
% motile sperm	73.49 $\pm$ 2.46	66.03 $\pm$ 0.5.49	13.19 $\pm$ 3.72*	7.07 $\pm$ 1.48*

* $p < 0.05$

Testicular levels of sorbitol dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G6PDH) were not affected after 55 days of treatment with 1 mg Cr(VI)/kg. Significant decreases ($p < 0.05$) were seen in these enzymes at the higher doses on treatment day 55, and at all doses on treatment day 90. The same pattern of significance was seen for γ-glutamyl transpeptidase (GGT) and LDH, excepting that activities of these enzymes were increased. Chromium levels in blood and testes were significantly increased ($p < 0.05$), in a dose dependent manner, for all doses at both time points.

The authors note that they set up their experiments to begin at weaning, when only spermatogonia and spermatocytes are present in the gonads, and end at 90 days of age, when the testis is presumed to be fully mature. The changes in activities of SDH, LDH, GGT, and G6PD were considered early biochemical markers of testicular damage. In particular, SDH and LDH were presumed to reflect germ cell damage while G6PD was taken to be a requirement for steroid biosynthesis and GGT to parallel Sertoli cell replication and maturation.

Ernst. (1990). Testicular toxicity following short-term exposure to tri- and hexavalent chromium: an experimental study in the rat.

Groups of eight adult male Wistar rats, of 100–120 days of age, were given Cr(VI), as sodium chromate, by i.p. injection in saline. Treatment was administered daily for five consecutive days with doses of 0, 1, 2, or 4 mg Cr(VI)/kg bw. The animals were sacrificed at seven and 60 days following cessation of treatment. Although it is not specified, it appears that four animals from each group were sacrificed at each of the two time points. Right side testes and epididymides were removed for evaluation.

No clinical symptoms of toxicity were observed in any group of rats. All the animals gained weight over the course of the study, but all Cr(VI) animals gained significantly less weight ($p<0.05$ for each dose) than controls, in what appeared to be a dose-dependent manner. Although the data are not shown, the paper reports that no testicular effects were observed at seven days post treatment for any of the dose groups. At 60 days post treatment, there were significant reductions in testes weights relative to body weight for all treated groups ($p<0.01$, compared to controls); the decreases occurred in a dose-dependent manner. According to the author's histological scoring system, there was a dose-dependent increase in the number of atrophic seminiferous tubules with loss of spermiogenic epithelium ($p<0.01$). At the high dose of 4 mg/kg, cellular organization was lost, with atrophic Leydig cells and degeneration in virtually all seminiferous tubules. Epididymal sperm counts were severely and significantly impacted ($p<0.01$), at all doses in a dose dependent manner. Sperm counts for all groups are shown in Table E7, below.

Table E7. Epididymal sperm counts ($\times 10^{-6}$) in rats given Cr(VI) by i.p. injection by Ernst (1990)

Dose mg/kg bw	**0**	**1**	**2**	**4**
Spermatozoa/epididymis ($\times 10^{-6}$)	640 $\pm$ 11	369 $\pm$ 12	131 $\pm$ 5	49 mg $\pm$ 7

It is worth noting that this study also contained experimental groups treated with trivalent chromium. Trivalent chromium-exposed animals also showed reduced weight gain relative to untreated controls, but did not show any evidence for testicular or epididymal toxicity. The author concludes that these data suggest the effects of Cr(VI) on male reproductive organs were specific, and not a result of systemic toxicity.

Mice, oral (drinking water)

Elbetieha and Al-Hamood. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility.

Adult male Swiss mice (50 days of age) were given 0, 1000, 2000, 4000, or 5000 mg potassium dichromate/L drinking water (ppm) for 12 weeks. At the end of treatment,

males were housed with two untreated, virgin female mice, for a period of ten days (presumed to encompass at least two estrous cycles). The females were sacrificed at one week following the end of mating for examination of their uterine contents. The numbers of (hexavalent chromium-treated) males and (untreated) females per group were as follows: 1) controls - 20 males, 40 females, 2) 1000 mg/L - 19 males, 38 females, 3) 2000 mg/L - 11 males, 22 females, 4) 4000 mg/L - 9 males, 18 females, 5) 5000 mg/L - 13 males, 26 females.

The frequency of pregnant females in each group ranged from 73% to 90.9%, and did not change significantly with dose. Both the low (1000 mg/L) and high (5000 mg/L) dose groups showed no significant effects on implantation frequency or the numbers of live fetuses. In contrast to controls and the other dose groups, however, these two groups both had resorptions (3 and 6, respectively; no statistical analysis). The numbers of implantations and live fetuses were significantly reduced in both the 2000 mg/L group ($p<0.005$ for both endpoints) and in the 4000 mg/L group ($p<0.05$ for both endpoints).

Table E8. Male reproductive toxicity data: Elbetieha and Al-Hamood, (1997)
Treated male mice (drinking water) mated with untreated females

Parameter	0	1000 mg/L	2000 mg/L	4000 mg/L	5000 mg/L
Pregnancy rate (%)	33/40 (82.5)	33/38 (86.8)	20/22 (90.9)	16/18 (88.8)	19/26 (73)
Live fetuses/litter	8.18 ± 1.59[a]	7.75 ± 1.80	6.33 ± 2.79**	6.86 ± 1.88*	7.15 ± 2.98
Implantations	8.18 ± 1.59	7.84 ± 1.56	6.33 ± 2.79**	6.86 ± 1.88*	7.84 ± 2.73
Resorptions	0	3	0	0	6

[a] Mean ± S.D.
*$p < 0.05$; **$p < 0.01$ (Student's *t* test)

Additional control (n=10) and treated (n=13/group, 2000 and 5000 ppm) animals were not mated, but were sacrificed at the end of the exposure period for determination of body and reproductive organ weights.

Compared to untreated controls, body weights were significantly reduced in both the 2000 and 5000 ppm groups ($p<0.01$ for both groups). Testes weights, relative to 10 g bw, were also significantly increased at both 2000 ppm ($p<0.01$) and 5000 ppm ($p<0.05$). Weights of seminal vesicles and preputial glands, both reported relative to 10 g bw, were significantly reduced at 5000 ppm potassium dichromate ($p<0.001$ for both endpoints).

The authors suggest that the litter effects seen with chromium exposure might result from increased peri-implantation mortality of fertilized ova. Additionally, the reductions in seminal vesicle and preputial gland weights might have resulted from effects on sex hormones.

Mice, oral (feed)

Al-Hamood et al. (1998). Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds.

Groups of 25 timed-pregnant BALB/c mice (plug day = day 0) were give 0 or 1000 ppm potassium dichromate in drinking water from gestation day 12 through lactation day 20. On the day of birth, litters were adjusted to eight pups each. At PND 60, each treated and control male was caged with two untreated, virgin female mice of the same strain. Females were left in mating cages for 10 days, to cover two complete estrous cycles. One week after the end of the mating period, the females were sacrificed for examination of their uterine contents.

No statistically significant change was found in fertility for males exposed to Cr(VI) pre- and postnatally. The litters they sired showed no significant differences from controls in the numbers of implantations, viable fetuses, or resorptions.

Additional males that were sacrificed on day 50 (14 controls, and 12 males exposed to Cr(VI) pre- and postnatally) revealed no differences between treated and control groups for body weight, testis weight, or seminal vesicle or preputial gland weights.

Zahid et al. (1990). Comparative effects of trivalent and hexavalent chromium on spermatogenesis of the mouse.

Male BABL/c albino Swiss mice were given potassium dichromate mixed into a powdered diet to Cr(VI) concentrations of 0, 100, 200, and 400 ppm. Seven animals were assigned to each concentration group. Treatment was continued for a total of 35 days. The animals were sacrificed at the end of the treatment period, and the right testis and epididymis removed for evaluation.

There were no significant differences among groups for the amount of food consumed or weight gained during the study. Mean testes and epididymides weights were also comparable among groups.

Histological examination of testes revealed significant increases in the percentages of degenerated tubules in treated animals as compared to controls ($p<0.001$ at each concentration) and in the percentages of undegenerated tubules without spermatogonia ($p<0.05$ at 100 ppm; $p<0.001$ at 200 and 400 ppm). The percentages of both effects increased with increasing Cr(VI) concentration.

Mean numbers of spermatogonia were significantly reduced in all three treated groups ($p<0.001$ at each concentration), in a concentration-dependent manner. Conversely, the mean numbers of resting spermatocytes were significantly increased over controls at all three dietary concentrations of Cr(VI) ($p<0.05$ for the 100 and 200 ppm groups, and $p<0.001$ for the 400 ppm group). The frequency of cells in meiotic leptotene did not

differ among groups, while significant increases in the frequency of cells in the pachytene phase of meiosis were seen at all three Cr(VI) concentrations ($p<0.001$ at each concentration). The frequency of cells in meiotic zygotene was significantly increased over controls in the 100 and 200 ppm groups ($p<0.01$ in each case), but not at 400 ppm Cr(VI).

Epididymal sperm counts were significantly reduced, relative to controls, in the 200 and 400 ppm Cr(VI) groups ($p<0.001$ for both concentrations). In these same two groups, the percentages of abnormal sperm were significantly increased ($p<0.001$ for both concentrations).

Rabbit, oral (gavage)

Yousef et al. (2006). Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits.

Four groups of six adult male New Zealand white rabbits were subjected to one of the following treatments: a) untreated controls, b) 8.3 μg folic acid /kg bw, c) 5 mg potassium dichromate/kg bw (contains 3.6 mg Cr(VI)), or d) 5 mg potassium dichromate/kg bw plus 8.3 μg folic acid /kg bw. The males were seven months of age and had a mean body weight of 2.873 kg at commencement of the study. Daily dosing for ten weeks was accomplished by "the aid of plastic tube directly inserted into the oropharyngeal region." During the treatment period, semen samples were collected weekly using an artificial vagina and a teaser doe. A total of 60 samples per treatment group were collected. Blood samples were collected every other week for determination of plasma testosterone levels. Animals were sacrificed at the end of the treatment period and testes and epididymides removed for examination.

No adverse clinical signs were observed during the treatment period for animals from any group. At the end of the treatment period, body weights in Cr(VI)-exposed group were significantly ($p<0.05$) lower than controls. When folic acid was given in combination with Cr(VI), however, mean body weight was significantly increased over the control value ($p<0.05$). Feed intake did not differ among groups. When considered as g/100 g bw, mean testis and epididymis weights were significantly reduced ($p<0.05$) as compared to controls. This effect was alleviated by the inclusion of folic acid. Mean plasma testosterone was significantly reduced in Cr(VI) exposed animals ($p<0.05$), while animals given Cr(VI) in combination with folic acid showed a significant increase in plasma testosterone ($p<0.05$). Analysis of variance (ANOVA) on the data found that body weight and plasma testosterone were both significantly ($p<0.001$) affected by both treatment and length of time on treatment, as well as by the interaction of treatment and time on treatment ($p<0.05$). The ANOVA also showed significant effects ($p<0.001$) of treatment on relative testes and epididymal weights.

Mean ejaculate volume was not affected by Cr(VI) exposure, but was significantly increased in folic acid-treated groups ($p<0.05$ whether or not Cr(VI) was also present).

Compared to controls, the Cr(VI)-treated group showed significant increases in reaction time (i.e., latency to full erection by the male after exposure to the female), pH, and the percentage of dead sperm ($P<0.05$ for each endpoint). This same group also showed statistically significant decreases in: packed sperm volume, sperm concentration, total sperm output, sperm motility, total motile sperm, percent normal sperm, total functional sperm fraction, and initial fructose ($p<0.05$ for all endpoints). The ANOVA indicated that all the above noted semen parameters, excepting ejaculate volume, were significantly affected by treatment and the interaction between treatment and time ($p<0.01$ for each endpoint). The ANOVA also found that sperm concentrations, total sperm output, sperm motility, total functional sperm fraction, packed sperm volume, initial fructose, and pH were all significantly ($p<0.01$) affected by time.

Seminal plasma parameters were also significantly affected by exposure to Cr(VI). Seminal plasma thiobarbituric acid-reactive substances (TBARS) were significantly elevated above control values in treated animals ($p<0.05$). At the same time, significant decreases were found for glutathione S-transferase (GST), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlP), and acid phosphatase (AcP) in Cr(VI)-treated versus control animals ($p<0.05$ in each case). By ANOVA, plasma TBARS was significantly affected by treatment and time ($p<0.01$), as were activities of GST, AST, ALT, AlP, and AcP ($p<0.01$ for each endpoint).

Total triglycerides, total protein, and total albumin in the seminal plasma were not significantly affected by exposure to Cr(VI). At the same time, total lipids, and glucose and urea concentrations in the seminal plasma were all significantly increased in Cr(VI)-exposed animals ($p<0.05$ for each). Total cholesterol, and both high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were significantly decreased in animals exposed to Cr(VI). The ANOVA results indicated that all of these parameters were significantly ($p<0.01$) affected by treatment, time, and the interaction of treatment and time.

The authors suggest that a Cr-induced reduction in plasma testosterone level might be at least partially the cause of observed declines in sperm concentration, total sperm output, and semen fructose. Additionally, the increased level to TBARS in Cr(VI)-exposed rabbits was taken to be evidence of overproduction of reactive oxygen species (ROS). In turn, the authors suggest ROS-mediated impairment of membrane fluidity and permeability of polyunsaturated membranes was a proximate cause of degraded semen quality. Concurrent administration of folic acid ameliorated, at least to some extent, the adverse effects of Cr(VI) on sperm and semen parameters. The authors suggest that the antioxidant effects of folic acid might have helped protect against oxidative damage caused by excess, Cr(VI)-induced levels of ROS.

Rabbit, i.p.

Behari et al. (1978). Comparative toxicity of trivalent and hexavalent chromium to rabbits. III. Biochemical and histological changes in testicular tissue.

Male rabbits, weighing approximately 1.5 kg, were divided into two treated groups of ten animals each, and one control group of eight animals. The treated groups were given i.p. injections of either 2 mg/kg body weight chromium nitrate (Cr(III)), or the same dose of potassium dichromate (Cr(VI)). Controls were given injections of saline vehicle only. Injections were given daily until sacrifice at either three or six weeks on treatment.

After three weeks of Cr(VI) treatment, testicular succinic dehydrogenase activity was significantly decreased ($p < 0.01$) relative to controls. Testicular activities of adenosine triphosphatase and acid phosphatase were not affected at this time point. After six weeks of Cr(VI) treatment, testicular levels of both succinic dehydrogenase and adenosine triphosphatase were significantly decreased relative to controls ($p < 0.01$, and $p < 0.001$, respectively), while acid phosphatase was still not affected.

No grossly observable changes were reported in the testes of any control or treated rabbits. Histopathological examination revealed "mild oedema of the interstitial tissue of the testes" of animals treated with Cr(VI) for 21 days. At the six-week exposure time point, this edema was more pronounced, and blood vessels appeared "congested." While the various cell types of the seminiferous epithelium were reported as appearing "almost normal," no spermatocytes were present.

The authors suggest that the inhibition of testicular succinic dehydrogenase and adenosine triphosphatase represented impaired energy metabolism in the cells, which in turn led to the degenerative changes observed in the seminiferous epithelium. The association between length of time on treatment and increasing enzyme inhibition, as well as evidence of progressive cellular damage, was taken to indicate that accumulation of the metal led to increasingly severe toxicity.

E.3. Integrative evaluation for male reproductive toxicity

E.3.1. Human data

A number of studies report on the potential impacts on male reproductive health related to exposure to stainless steel welding with its Cr(VI) fumes. Effects on sperm quality and fecundability, infertility, and male-mediated spontaneous abortion in couples were studied. In addition, one study considered sperm quality in men working in a chromate factory (Kumar et al., 2005). The design and results of these studies on semen quality are given in Table E9.

Table E9. Semen Quality Among Workers Exposed to Hexavalent Chromium

Authors / Study Design / Location	**Study participants / Exposure or outcome groups**	**Measures of effect (95% CI)**	**Comments**
Jelnes and Knudsen, 1988 Cross-sectional design Denmark	SS manufacturing SS welders (n=77) Non-welders (n=68)	No difference in semen quality parameters	Matched on age and tobacco use. Blood and urine chromium levels were measured but not reported.
Mortensen, 1988 Case-control design Denmark	Hospitals in 4 cities Cases: Men with abnormal sperm based on specified parameters (n=828) Controls: Men whose sperm did not meet specified parameters (n=1689)	OR for poor sperm quality in SS welders: 2.34 (0.95 – 5.73)	OR adjusted for age, tobacco and alcohol use, and other factors [logistic regression analysis]
Bonde 1990a; Bonde and Christensen, 1991; Bonde and Ernst, 1992 Cross-sectional design Denmark	Six workplaces in Aalborg SS welders (n=35) Cr level in urine[1]: 2.07±1.03 nmol/nmol creatinine Nonwelding metal workers (n=32) chromium level in urine[1]: 0.76±0.49 Electricians (n=22) chromium level in urine[1]: 0.68±0.23	Decreased sperm count* Decreased semen volume* Increased percentage of subjects with immature sperm forms* Decreased proportion of motile sperm* Increased percentage of sperm with poor to no motility** Decreased testosterone*	Age, tobacco and alcohol use, and other potential confounders were addressed in multiple regression analyses. Because of a focus on effects in SS versus mild steel welders, the results comparing SS welders to nonexposed groups are not highlighted in the reports.
Bonde 1990b; Bonde and Ernst, 1992 Cross-sectional design Denmark	Six workplaces in Aalborg SS welders (n=18) before and after a 3-week vacation	Post-vacation mean change in percentage normal sperm forms (SD): 3 weeks: +1.7 (5.3) 5 weeks: +4.8 (4.7) 8 weeks: +3.7 (2.7)	Improvement was not statistically significant in the SS welders; in the same parameter, referents had a significant increase over time
Hjollund et al., 1998a Cohort design Denmark	Couples planning 1st pregnancy, from metal worker and other unions, recruited by mail Paternal occupation SS welders (n=29) Metal workers not currently welding (n=68) Non-metal workers (n=200)	No differences in semen quality parameters	No differences were seen in urine chromium levels measured in welders and metal workers not currently welding, nor in pre- versus post-shift samples. Control for potential confounders did not change results.

Table E9. (continued)

Authors / Study Design / Location	Study participants / Exposure or outcome groups	Measures of effect (95% CI)	Comments
Tielemans et al., 1999 Case-control design The Netherlands	Two infertility clinics Cases: Men with abnormal sperm based on specified parameters (n=692) Controls: Men whose sperm did not meet specified parameters (n=207)	OR for SS welding: 0.53 (0.21 – 1.37)	OR adjusted for a range of factors
Li et al., 1999; *Li et al., 2001* Cross-sectional design China	Electroplating (n=21), Avg. Cr(VI) = 0.235 mg/m^3 Unexposed (n=22) Avg. Cr(VI) = 0.017 mg/m^3	Decreased sperm count* Decreased percentage sperm with normal mobility*	No controlling for tobacco or alcohol use or other potential confounders
Danadevi et al., 2003 Cross-sectional design India	Welders (unspecified type) (n=28) Avg. blood chromium level = 131.0 µg/l Non-welders (n=27) Avg. blood chromium level = 17.4 µg/l	Decreased sperm count**** Reduced progressive sperm motility**** Reduced vitality**** Abnormal sperm morphology****	Matched on age, tobacco and alcohol use, and economic status Chromium blood levels significantly correlated with impacts on sperm parameters
Kumar et al., 2005 Cross-sectional design India	Chromate factory (n=61) Avg. blood chromium level = 63.7 µg/l Nonexposed (n=15) Avg. blood chromium level = 22.8 µg/l	Abnormal sperm morphology***	No controlling for tobacco or alcohol use or other potential confounders

Abbreviations: Avg, average; CI, confidence interval; Cr, chromium; CrVI, hexavalent chromium; n, number of subjects; OR, odds ratio; SD, standard deviation; SS, stainless steel;
*p<0.05; **p<0.01; ***p<0.005; ****p<0.001
1. Chromium in urine was measured in a subset of these workers, including 24 SS welders, 12 nonwelding metal workers, and 16 electricians.

To find an effect of Cr(VI) on these endpoints, if it exists, the exposed Cr(VI) groups must have exposures large enough and different enough from the exposure of the comparison group(s), and the exposure indicators selected must generally reflect these differences. While each study has limitations in this regard, the studies for sperm quality endpoints that appear to come closest to achieving this include: the series of studies conducted by Bonde and collaborators (Bonde, 1990a; Bonde, 1990b; Bonde and Christensen, 1991; Bonde and Ernst, 1992a; Bonde, 1993) in six workplaces in Aalborg, Denmark; the series of studies by Hjollund *et al.* (Hjollund et al., 1998a; Hjollund et al.,

1998b; Hjollund et al., 2000) of couples planning their first pregnancy, recruited from metal worker and other unions via mail; the studies of Chinese workers by Li et al. (Li et al., 1999; Li et al., 2001) and the studies by Danadevi et al. (2003) and Kumar et al. (2005) in India. Each of these efforts included measurement of chromium in either ambient air in the workplace or in urine or blood of workers (in some cases both air and biological measurements were made), providing some basis for evaluating the assumption that differences in Cr(VI) exposure existed between the groups being compared, although as noted in Section E1, the specificity of the indicators to Cr(VI) is fairly limited, with erythrocyte levels followed by whole blood being better Cr(VI) indicators than other measures employed. Still, for the two study groups that included ambient air measurements, the levels appear to generally indicate Cr(VI) exposure potential with exposed men in the Li et al. (2001) study experiencing much higher Cr(VI) levels (235 $\mu g/m^3$) and "unexposed" men in the study (17 $\mu g/m^3$) or the SS welders in the Bonde studies (2.0 $\mu g/m^3$). Given this variation in exposures experienced in different work environments, variation in results in these studies is expected.

The Bonde (1990a) study in Denmark and the Li et al. study both found significant decreases in sperm count and percentage of sperm with normal mobility when comparing those with higher to those with lower chromium exposure. The Li et al. study, despite its higher exposures, was limited by the lack of control for potentially important confounders. In the Bonde (1990a) study, potential confounders were addressed in the analyses. Additional effects on semen quality in SS welders in comparison to unexposed workers were identified by this study. Similarly, in the Danadevi et al. (2003) study in India, where the workers had relatively high levels of exposure to chromium and the study addressed potential confounders through matching, significantly decreased sperm count, reduced progressive sperm motility, and increased abnormal sperm morphology were observed; in this study, blood levels of chromium were significantly correlated with impacts on sperm parameters. The Bonde (1990a) study failed to find such a correlation, but this may be due to the substantially lower exposures experienced by the Danish workers; the blood levels were measured in these workers prior to beginning work (Monday morning). The two studies also reported blood levels in different units so, to allow direct comparison, OEHHA has also converted the value in Bonde (1990a) to the units used by Danadevi et al. (2003) (mean values: Bonde 1990a, 17.3 nmol/l (0.9 µg/l) in the most highly exposed group; Danadevi et al., 2003, 131 µg/l in welders).

Some of the studies did not find a difference in semen quality or other effects on male reproductive health. Most of these lacked information that would substantiate the exposure characterization of the groups being compared. For example, in studies that defined the "exposed" group as those who had ever welded stainless steel for one year or more, individuals with substantial chromium exposure (for example, daily SS welding for five or more years) were grouped with others who may have welded SS only occasionally for no more than one year. Examples of these studies include Mortensen (1988), Tielemans et al. (1999), Bonde et al. (1990c), Bonde et al. (1992b), and Hjollund et al. (1995). Hjollund et al. (2005) gathered slightly more information on SS welding from subjects in their study, but found the participants had very little exposure to SS welding (85% welded SS less than one hour per day). A couple of these studies did find

indications of an effect of SS welding on semen quality (Mortensen, 1988) and male-mediated spontaneous abortion (Bonde, 1990b). Of those that did not, both Tielemans et al. (1999) and Hjollund et al. (2005) had study populations drawn from those experiencing fertility problems (an infertility clinic and couples who had undergone *in vitro* fertilization, respectively), who may not be representative of the population at large. An early study (Jelnes and Knudsen, 1988) that found no effect of SS welding on semen quality parameters apparently measured chromium in blood and urine but did not report the results; they do, however, report that their comparison group of nonwelders was exposed to "generally high" levels of metal dust in these SS manufacturing plants and thus was a suboptimal reference population.

Studies examining effects of Cr(VI) on fecundability, infertility, and male-mediated spontaneous abortion in couples are given in Table E10. The series of studies by Hjollund et al. (Hjollund et al., 1998a; Hjollund et al., 1998b; Hjollund et al., 2000) of couples planning their first pregnancy, recruited from metal worker and other unions via mail, presents the best examination of potential effects with both biological measurements to distinguish exposure groups and control of confounders of concern. The lack of a finding of effect on semen quality in relation to exposure status may also be a reflection of the relatively low exposure of the study base, according to the authors (Hjollund et al., 1998a). The biological measurements are not reported in any detail, and those that are, based on small numbers of participants (e.g., SS welders, n=6) show no differences between welders and either comparison group (nonwelders or nonmetal workers), or pre- versus post-shift chromium levels in the urine samples (Hjollund et al., 1998b). The probability of conceiving in a given menstrual cycle (fecundability) in these couples was decreased with current SS welding exposure, through the results were not statistically significant (OR=0.82, 95% CI 0.45 – 1.50) (Hjollund et al., 1998b). Fecundability in these couples also decreased with increasing years of SS welding, although again without statistical significance.

Table E10. Fecundability, Infertility and Male-Mediated Spontaneous Abortion in Couples that included a Male Stainless Steel Welder

Authors / Study Design / Location	**Source of study participants / Exposure or outcome groups**	**Measures of effect (95% CI)**	**Comments**
Bonde et al., 1990c Cohort design Denmark	National pension records, born ≥1945 Men who worked ≥1 year as welders, at least some SS (n=2283 ever SS welders) Comparison to same men during years they were not welding	*Fertility* Probability of spouse having a child during year man was welding Any welding: RR=0.91* (0.85 – 0.98) Only men who ever welded SS RR=0.95 (0.90 – 1.01)	Analyses restricted to men who ever welded SS also included some adjustment for different exposure potential (TIG =1, MMA=2) Comparison to years the same men weren't welding assumes effect is short-lived
Bonde et al., !992b Cohort design Denmark	National pension records, born ≥1945 Pregnancies in spouses of men who worked ≥1 year as welders (n=2283 ever SS welders) Comparison to pregnancies in spouses of the same men during years they were not welding	*Male-mediated spontaneous abortion* - Pregnancies at risk from any paternal SS welding OR= 2.0 (1.1 – 3.5) * - Paternal MMA SS welding OR=1.99 (1.07 – 3.69) * - Paternal TIG SS welding OR=1.71 (0.84 – 3.39) Exposure-response trend (p=0.022)	Analysis of spontaneous abortion restricted to pregnancies that preceded a recorded birth in the same spouse. The pregnancy was considered at risk of paternal welding exposure if the subsequent birth was at risk.
Bonde 1993 Case-control Denmark	Six workplaces in Aalborg Men reporting infertility on questionnaire (n=52) Men reporting no infertility (n=208)	*Infertility among those with any welding (MS or SS) exposure* OR=2.2 (1.1 – 4.6) *	Infertile men were age-matched to those who did not report infertility. Infertility defined as no pregnancy after ≥2 years of trying. No details provided on those welding SS only.
Hjollund et al., 1995 Cohort design Denmark	National pension records, born ≥1945 Pregnancies (n=862) in spouses of men who worked ≥1 year as SS welders Pregnancies (n= 1037) in spouses of the same men during years they were not welding	*Male-mediated spontaneous abortion* - Pregnancies at risk from any paternal SS welding OR= 0.78 (0.55 – 1.1)	Re-analysis of the cohort reported in Bonde et al., 1992b. For more accurate dating of event, used a different source of information (hospital registry) on occurrence of spontaneous abortions. Missed some early pregnancy loss.

Table E10. (continued)

Hjollund et al., 1998b Cohort design Denmark	Couples planning 1st pregnancy, from metal worker and other unions, recruited by mail Paternal occupation SS welders (n=29) Nonwelders (n=71) Nonmetal workers (n=205)	*Fecundability* Current exposure to male SS welding OR=0.82 (0.45 – 1.50) Historical SS welding compared to nonmetal workers ≤1 yr: OR=1.73 (1.01 – 2.97) 2 -5 yr: OR=1.00 (0.53 – 1.86) 6+yr: OR =0.56 (0.26 – 1.19)	Fecundability was defined as the probability of conceiving in a given menstrual cycle. Because of a significant inter-action with male smoking when comparing welders to nonwelding metal workers, comparison is shown for external group (nonmetal workers).
Hjollund et al., 2000 Cohort design Denmark	Couples planning 1st pregnancy, from metal worker and other unions, recruited by mail Paternal occupation SS welders (n=29) Nonwelders (n=71) Nonmetal workers (n=205)	*Male-mediated spontaneous abortion* Pregnancies with current paternal SS welding RR=2.6 (1.2 – 5.5) Pregnancies in relation to years of SS welding 1-5 yr, RR=1.2 (0.4 – 3.3) >5 yr, RR=2.6 (1.1 – 6.1)	Both subclinical and clinically diagnosed spontaneous abortions were included. Exposure information collected prior to pregnancy outcome. Subclinical pregnancies detected by measuring hCG in urine.
Hjollund et al., 2005 Cohort design Denmark	*In vitro* fertilization registry Paternal occupation SS welders (n=91) Not exposed to welding (n=2925)	*Male-mediated spontaneous abortion* Pregnancies with male partner exposed to SS welding RR=0.59 (0.36 – 0.98)	The majority (85%) of men welding SS did so <1 hour per day

Abbreviations: CI, confidence interval; hCG, human chorionic gonadotropin; MMA, manual metal arc; n, number of subjects; OR, odds ratio; RR, risk ratio; SS, stainless steel; TIG, tungsten inert gas.
*p<0.05

The examination of male-mediated spontaneous abortion by this group (Hjollund et al., 2000) has several strengths First, this study definitively and prospectively established the onset of pregnancy. This was achieved through the collection of urine by female participants during ten consecutive days from the onset of each period of vaginal bleeding and stored in home freezers for each of the menstrual cycles per participant included in the study. Measurement of hCG in these urine samples allowed identification of early pregnancy losses. Information on the outcome of pregnancies that were clinically diagnosed was achieved for 100% of the couples. The outcome of interest, spontaneous abortion, was determined for all participants equally, regardless of exposure status. The rate ratio for spontaneous abortion with current paternal SS welding was significantly increased (rate ratio=2.6, 95% CI 1.2 – 5.5). In addition, examining pregnancies in relation to years of SS welding provided an indication of increasing risk with increasing years of SS welding (1-5 years, rate ratio=1.2, 95% CI 0.4 – 3.3; >5

years, rate ratio=2.6, 95% CI 1.1 – 6.1). This study indicates the negative impacts on male reproductive success as a result of chromium exposure from SS welding, and also point to another explanation for the limited findings in earlier studies that failed to consider years of SS welding in assigning exposure status to subjects.

In summary, a number of studies showed impacts on male reproductive parameters from occupational exposure to Cr(VI) (primarily through SS welding). Study limitations especially with regard to establishing groups with clear differences in exposure status appear to explain studies with negative findings. The studies with the higher exposures or better designs did find increased risks of adverse outcomes in men with Cr(VI) exposure.

E.3.2. Animal data

Available studies on the potential male reproductive toxicity of Cr(VI) in experimental animals include four studies in monkeys performed by the oral route (drinking water) in monkeys (Aruldhas et al., 2004; Aruldhas et al., 2005; Aruldhas et al., 2006; Subramanian et al., 2006). Of seven studies conducted in rats, one provided Cr(VI) in drinking water (Bataineh et al., 1997), two were performed by feed or gavage (Chowdhury and Mitra, 1995; Li et al., 2001), and the remaining four by i.p. injection (Ernst, 1990; Saxena et al., 1990b; Murthy et al., 1991; Ernst and Bonde, 1992). Three mouse studies were conducted by the oral route of exposure, one by drinking water (Elbetieha and Al-Hamood, 1997), and two by feed (Zahid et al., 1990; Al-Hamood et al., 1998). Of two studies in rabbits, one was conducted by gavage (Yousef et al., 2006) and the other by i.p. injection (Behari et al., 1978). Relevant data from these studies are summarized in Table E11.

Table E11. Animal studies of male reproductive toxicity

Reference	Study design	Systemic toxicity	Male reproductive toxicity
Subramanian et al. (2006)	Macaque, drinking water 0. 50, 100, 200, 400 ppm; 6 months potassium dichromate 3 ♂/group	Concentrations > 400 ppm caused death within 3 months	↓ sperm counts & motility, at ≥ 100 ppm ↓ superoxide dismutase & catalase activities in seminal plasma & sperm at ≥ 100 ppm ↑ reduced glutathione & H_2O_2 in seminal plasma & sperm at ≥ 100 ppm
Aruldhas et al. (2006)	Macaque, drinking water 0, 100, 200, 400 ppm; 180 days potassium dichromate 3 ♂/group	No data reported	Histological evidence for phagocytosed sperm at all concentrations (no statistical analysis presented)

Table E11. (continued)

Aruldhas et al. (2005)	Macaque, drinking water 0, 100, 200, 400 ppm; 180 days potassium dichromate 6 ♂/group	No data reported	Testicular damage apparent at light and TEM levels, all concentrations ↓ testicular superoxide dismutase, catalase, glutathione perioxidase, glutathione reductase, & glucose-6-phosphate dehydrogenase activities, all concentrations ↓ testicular γ-glutamyl transpeptidase activity at 400 ppm ↑ reduced glutathione & H_2O_2 concentrations ↓ testicular concentrations of vitamins C, A, & E
Aruldhas et al. (2004)	Macaque, drinking water 0, 100, 200, 400 ppm; 180 days potassium dichromate 3 ♂/group	No data reported	Microscopic evidence of epididymal epithelium pathology, all concentrations, but not quantified
Bataineh et al. (1997)	Rat, drinking water 0, 1000 ppm; 12 weeks potassium dichromate 12-13 ♂/group	Animals reported to remain health	↓ number mounts & % ♂'s ejaculating No effect on fertility with 10 day mating period ↑ ejaculatory latency & post-ejaculation intervals ↓ aggressive behaviors and fights ↓ body wts, testes wts, seminal vessicle wts, & preputial gland wts
Li, et al. (2001)	Rat, feed or gavage 0, 10, 20 mg/kg-day; 6 days (evaluated 6 weeks later) CrO_3 8-11 ♂/group	No data reported	↓ epididymal sperm counts, both doses ↑ abnormal sperm, & abnormal histology, both doses
Chowdhury & Mitra (1995)	Rat, gavage 0, 20, 40, 60 mg/kg-day; 90 days sodium dichromate 10 ♂/group	↓ body wt & % wt gain, 40 & 60 mg/kg-day (no statistics)	↓ testes wts, 40 & 60 mg/kg-day ↓ testicular protein concentration, all doses ↓ testicular DNA & RNA contents, seminiferous tubule diameter, Leydig cell populations & Leydig cell nuclear diameters, 40 & 60 mg/kg-day Relative to 8 Sertoli cells: ↓ pachytene spermatocyte count, & stage-7 spermatid count 40 & 60 mg/kg-day ↓ resting spermatocyte count, 60 mg/kg-day ↑ testicular cholesterol, 40 & 60 mg/kg-day ↓ testicular succinic dehydrogenase, 40 & 60 mg/kg-day ↓ testicular $3\beta\Delta^5$-HSH, serum testosterone, all doses

Table E11. (continued)

Ernst & Bonde (1992)	Rat, i.p. 0, 0.05 mg/kg^{-1}; 5 days/wk, 8 wks sodium chromate 2 control & 2 treated groups, 10 ♂/group 1 treated & 1 control group sacrificed for exam at the end of treatment, the others after 8-wk recovery	No clinical symptoms No differences between groups in body weights	No significant effect on sperm counts or frequency of abnormal sperm, w or w/o recovery period ↓ % motile sperm w/o recovery period ↓ testosterone, ↑ FSH & LH w/o recovery ↓ LH w recovery
Murthy et al. (1991)	Rat, i.p. 0, 2 mg/kg-day; 15 days potassium dichromate 8 ♂/group	No effects on body weight, and feed or water consumption	No effects on sperm counts or motility ↑ blood and testicular chromium levels Histopathological evidence for changes in Sertoli cell membrane properties
Saxena et al. (1990b)	Rat, i.p. 0, 1, 2, 3 mg/kg; daily from weaning to PND 55 or 90 (sacrifice) potassium dichromate 15 ♂/group	0/15, 0/15, 2/15, 3/15 deaths in each dose group, respectively ↓ bw at 2 & 3 mg/kg (no statistics)	↓ absolute, but not relative, testes wts at 2 & 3 mg/kg, both time points No histopathological changes evident at 55 days At 90 days, abnormal testicular pathology, all doses ↓ epididymal sperm counts & sperm motility, 2 & 3 mg/kg ↓ testicular levels of SDH & G6PDH at 3 mg/kg on PND 55, and all doses at PND 90 ↑ testicular GGT & LDH at 3 mg/kg on PND 55, and all doses at PND 90
Ernst (1990)	Rat, i.p. 0, 1, 2, 4 mg/kg-day; 5 days sodium chromate 8 ♂/group; sacrifice at 7 and 60 days post treatment	No clinical symptoms ↓ wt gain, all doses	No testicular effects at 7 days post-treatment At 60 days post-treatment: ↓ testes/bw, all doses ↑ histopathological findings w ↑ dose ↓ epididymal sperm counts, all doses
Elbetieha & Al-Hamood (1997)	Mouse, oral, drinking water 0, 1000, 2000, 4000, 5000 ppm; 12 weeks prior to mating with untreated ♀ potassium dichromate 9-20 ♂/group	↓ bw at 2000 & 5000 ppm (other doses not in this experiment)	No effect on pregnancy rate ↓ implantations and live fetuses at 2000 & 4000 ppm ↑ resorptions at 1000 & 5000 ppm ↑ testes wts at 2000 & 5000 ppm (other doses not in this experiment) ↓ wts of seminal vesicles & preputial glands, 5000 ppm
Al-Hamood et al. (1998)	Mouse, oral, feed 0, 1000 ppm; gd12-lactation potassium dichromate 25 ♂/group; mated with untreated ♀ on PND 60	No effect on bw	No effect on fertility or litter parameters No effect on testes wt, seminal vesicle wt, or preputial gland wt

Table E11. (continued)

Zahid et al. (1990)	Mouse, oral, feed 0, 100, 200, 400 ppm; 35 days potassium dichromate 7 ♂/group	No effects on feed consumption or wt gain	No effects on testis or epididymal wts ↑ abnormal testicular histology, all concentrations ↓ spermatogonia, all concentrations ↑ mean numbers of resting spermatocytes, all concentrations ↓ epididymal sperm counts, 200 & 400 ppm ↑ % abnormal sperm, 200 & 400 ppm
Yousef, et al. (2006)	Rabbit, oral, gavage 0 or 5 mg/kg bw, w or w/o 8.3 mg/kg bw folic acid; daily for 10 weeks potassium dichromate 6 ♂/group	No clinical symptoms ↓ bw, which was alleviated by folic acid No effect on feed intake	↓ testis and epididymis wt relative to bw, which was alleviated by folic acid ↓ testosterone, alleviated by folic acid ↑ reaction time, semen pH, & % dead sperm No effect on ejaculate volume ↓ packed sperm volume, sperm concentration, total sperm output, sperm motility, total motile sperm, % normal sperm, total functional sperm fraction, & initial fructose ↑ seminal plasma TBARS ↓ GST, AST, AlP, & AcP ↑ total lipids, glucose, & urea concentrations in seminal plasma ↓ total cholesterol, HDL-C, & LDL-C
Behari et al. (1978)	Rabbit, i.p. 0, 2 mg/kg-day; 3 or 6 weeks potassium dichromate 8-10 ♂/group	No mortality or morbidity	At 3 weeks: ↓ testicular succinic dehydrogenase Histopathological changes At 6 weeks: ↓ testicular succinic dehydrogenase & adenosine triphosphatase Abnormal histology more extensive and severe than at 3 weeks

All but one of the 16 studies showed adverse effects on some aspect of the male reproductive system, regardless of species or whether Cr(VI) was given in drinking water, in feed, by gavage, or by i.p. injection. Observations included histopathology findings (Behari et al., 1978; Ernst, 1990; Zahid et al., 1990; Saxena et al., 1990b; Murthy et al., 1991; Chowdhury and Mitra, 1995; Li et al., 2001; Aruldhas et al., 2004; Aruldhas et al., 2005; Aruldhas et al., 2006), altered sperm parameters (Ernst, 1990; Zahid et al., 1990; Saxena et al., 1990b; Ernst and Bonde, 1992; Li et al., 2001; Subramanian et al., 2006; Yousef et al., 2006), altered testicular biochemistry (Behari et al., 1978; Saxena et al., 1990b; Chowdhury and Mitra, 1995; Aruldhas et al., 2005; Subramanian et al., 2006; Yousef et al., 2006), altered sexual and aggressive behavior (Bataineh et al., 1997), altered weights of testes, epididymides, and/or other accessory organs (Ernst, 1990; Zahid et al., 1990; Saxena et al., 1990b; Chowdhury and Mitra, 1995; Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997), decreases in testicular protein, DNA, and RNA (Chowdhury and Mitra, 1995), and decreased serum and/or testicular testosterone (Zahid et al., 1990; Ernst and Bonde, 1992).

The only study that did not report evidence of male reproductive toxicity was by Al-Hamood et al. (1998) in male mice exposed via drinking water. This study commenced

in utero, as their dams were treated from gestation day 12 through lactation. At a postnatal age of 60 days, treated and control males were mated with untreated females. No effects on fertility or the weights of male reproductive organs were observed. The study did not examine fertility with shorter mating opportunities, or examine the reproductive organs at time points during, or immediately following, exposure.

Fertility was only tested in a three studies (Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997; Al-Hamood et al., 1998). Two of these studies (Bataineh et al., 1997; Al-Hamood et al., 1998) reported no adverse effects on fertility. Elbetieha and Al-Hamood (1997) found no effects on pregnancy rate among untreated female mice mated to exposed males, but did reportdecreases in implantation frequency and numbers of live fetuses per litter, as well as increases in resorptions.

Morphological damage, observed by light microscopy and/or at the ultrastructural level, was described following Cr(VI) exposure in non-human primates (Aruldhas et al., 2004; Aruldhas et al., 2005; Aruldhas et al., 2006), rats (Ernst, 1990; Saxena et al., 1990b; Murthy et al., 1991; Chowdhury and Mitra, 1995; Li et al., 2001), mice (Zahid et al., 1990), and rabbits (Behari et al., 1978). Specific changes included, but were not limited to: phagocytosed sperm (Aruldhas et al., 2006), disorganized seminiferous tubules (Aruldhas et al., 2005), damage to the epididymal epithelium(Aruldhas et al., 2004), damage to Sertoli cell membranes (Murthy et al., 1991), decreased seminiferous tubule diameter (Saxena et al., 1990b; Li et al., 2001), atrophic seminiferous tubules with loss of spermiogenic epithelium (Ernst, 1990), “degenerated” tubules as well as “undegenerated” tubules devoid of spermatogonia (Zahid et al., 1990), and edema of testicular interstitial tissues (Behari et al., 1978).

In macaques, evidence of testicular damage was reported at concentrations of Cr(VI) in drinking water of as low as 100 ppm, with additional and increasingly severe effects at concentrations of 200 and 400 ppm (Aruldhas et al., 2004; Aruldhas et al., 2005; Aruldhas et al., 2006; Subramanian et al., 2006). A concentration of 50 ppm was tested in only one study, Subramanian et al. (2006), and was without reported effects; the same study mentioned that pilot work (data not presented) found concentrations greater than 400 ppm to be lethal. In rats, the only drinking water study (Bataineh et al., 1997), tested a single concentration of 1000 ppm, which was associated with male reproductive effects; no mortality was reported. Two drinking water studies in mice (Elbetieha and Al-Hamood, 1997; Al-Hamood et al., 1998) did not find evidence of testicular toxicity with Cr(VI) at a concentration of 1000 ppm. Higher concentrations of 2000, 4000, and 5000 ppm were associated with male reproductive toxicity (Elbetieha and Al-Hamood, 1997).

In rat feed or gavage studies, adverse effects on the male reproductive system were seen with doses of Cr(VI) ranging from 10–60 mg/kg-day (Chowdhury and Mitra, 1995; Li et al., 2001). The one feeding study conducted in mice (Zahid et al., 1990), reported an association between Cr(VI) concentrations of 100–400 ppm and male reproductive effects. In rabbits, the single gavage study (Yousef et al., 2006) found male reproductive effects at the test dose of 5 mg/kg-day.

When given by the i.p. route, rats showed testicular effects at doses of Cr(VI) ranging from 0.05–4 mg/kg-day (Ernst, 1990; Saxena et al., 1990b; Murthy et al., 1991; Ernst and Bonde, 1992). In rabbits, the i.p. route was associated with adverse effects on male reproductive organs at a dose of 2 mg Cr(VI)/kg-day (Behari et al., 1978).

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G. APPENDIX

Acronyms

AcP:	acid phosphatase
AI:	adequate intake
AlP:	alkaline phosphatase
ALT:	alanine aminotransferase
AST:	aspartate aminotransferase
bw:	body weight
Cal EPA:	California Environmental Protection Agency
CDHS:	California Department of Health Services
CI:	confidence intervals
Cr(III):	trivalent chromium
Cr(VI):	hexavalent chromium
CRL:	crown-rump length
E_2:	estradiol
EAR:	estimated average requirement
EDs:	enumeration districts
FSH:	follicle-stimulating hormone
G6PDH:	glucose-6-phosphate dehydrogenase
GD:	gestation days
GGT :	γ-glutamyl transferase
GH:	growth hormone
GST :	gluathione s-transferase
HAS:	high alloy steel
hCG:	human chorionic gonadotropin
H_2O_2 :	hydrogen peroxide
ICP-MS:	inductively coupled mass spectrometer
IARC:	International Agency for Research on Cancer
i.p.:	intraperitoneal
i.v.:	intravenous
IVF:	in vitro fertilization
LOAEL:	lowest observed adverse effect level
LF :	lipofuscian
LDH:	lactate dehydrogenase
LDH-x:	lactate dehydrogenase C4 isoenzyme
LH :	leutinizing hormone
MMA:	manual metal arc
MN PCE:	micronucleated polychromatic erythrocytes
MS:	mild steel
NCE:	normochromatic erythocytes
NAS:	National Academy of Sciences
NTP:	National Toxicology Program
P_4:	progesterone
ppm:	parts per million

PND:	postnatal day
PRL :	prolactin
OR:	odds ratio
RBC:	red blood cells
ROS:	reactive oxygen species
RR:	relative risk
sc:	subcutaneous
SDH :	sorbitol dehydrogenase
SIGC:	spontaneously immortalized rat granulose cell line
SOD :	superoxide dismutase
SS:	stainless steel
T:	testosterone
TBARS :	thiobarbituric acid-reactive substances
TEM :	transmission electron microscopy
TIG:	tungsten inert gas
wts:	weights